EXHIBIT A

SMITH KLINE & FRENCH LABORATORIES LIMITED and SMITHKLINE BEECHAM CORPORATION d/b/a GLAXOSMITHKLINE, Plaintiffs, v. TEVA PHARMACEUTICALS USA, INC., Defendant.	)	Civil Action No. 05-197-GMS

EXPERT REPORT OF EGON E. BERG

1. I am an attorney specializing in the area of patent and trademark law. I am a member of the New York and District of Columbia Bars and have been admitted to practice before the United States Patent & Trademark Office ("Patent Office") for over forty years.

2. I have been retained by Wilmer Cutler Pickering Hale and Dorr LLP, on behalf of the Plaintiff GlaxoSmithKline ("GSK"), to provide my opinions regarding the Defendant Teva Pharmaceutical USA, Inc.'s ("Teva") allegations of inequitable conduct in this case.

3. I am being compensated for my time spent in connection with rendering my opinions in this case at my customary rate of $350 per hour. My compensation is not contingent in any way upon the conclusions or opinions I reach, any testimony I may give, or the outcome of this case.

4. My curriculum vitae describing my educational background and work experience is attached as Attachment A to this report.

5. In the last four years, I have not been deposed, nor have I testified at trial as an expert.

6. This report summarizes my opinions regarding the topics for which I have been asked by GSK to offer expert testimony, as well as the basis for my opinions. I reserve the right to revise this report as additional information becomes available.

7. For a list of documents and sources that I have considered in formulating my opinion in this case, please see Attachment B.

SUMMARY

8. I have been asked to offer my opinions regarding two inequitable conduct issues in this case in light of the standard practices of lawyers who draft patent applications and the patent examiners who review them. Because of my varied background as a patent examiner at the United States Patent & Trademark Office (with a focus in organic chemistry), a private practitioner, and an attorney with thirty-three years of experience at a pharmaceutical corporation, I believe I can bring unique perspective to the standard practices in the field in which I have actively participated for nearly forty-seven years. I continue to be active in the field as I currently consult for large and small pharmaceutical companies.

9. Based upon this experience and upon certain factual assumptions that have been provided to me, it is my opinion that there is no basis for a finding of inequitable conduct with respect to the scope of the generic claims presented by the applicant to the patent examiner in the United States Patent & Trademark Office for examination, and

ultimately allowed by the patent examiner in U.S. Patent Nos. 4,452,808 (the "'808 patent") and 4,824,860 (the "'860 patent") or based on the information in the '808 patent specification concerning human dosage.

10. With respect to the generic claims of the '808 and '860 patents, it is my opinion that their scope is reasonable in light of the patents' specification disclosures and that such claim scope is typical of generic claims that patent practitioners appearing before the United States Patent & Trademark Office routinely seek to protect an invention to a new chemical compound and/or to a method of using that compound. It is a function of a patent attorney or patent agent to seek patent claims as broad as possible in light of the patent application disclosure taking into account input from the inventor(s), the relevant prior art of which he or she is aware, and the conditions for patentability defined in the patent statute, 35 U.S.C § 101, et. seq. It is up to the patent examiner whether to allow or deny any given claim scope. Teva does not appear to challenge the accuracy of the description of the work leading to the inventions of the '808 patent and the '860 patent set forth in the specifications of these two patents. In the absence of the non-disclosure or material misrepresentation of material information, there is no basis for inequitable conduct.

11. With respect to the information in the '808 patent specification concerning human dosage, the plain language of this example indicates that it is a prophetic example. Moreover, a patent examiner would construe it as such in light of the language used and the commonly known fact that nearly all pharmaceutical patents which claim new chemical entities ("NCE") are filed very early before human clinical trials are commenced. It is generally known that clinical trials are begun many years after the

filing of an initial patent application for a NCE. Accordingly, there is no basis for concluding that there was any misrepresentation in the '808 patent with respect to dosage.

QUALIFICATIONS

12. I received a Bachelors of Science degree in Chemistry from Rutgers University in 1959, and a *Juris Doctor*, with Honors, from the George Washington University School of Law in 1963.

13. I was a patent examiner at the United States Patent & Trademark Office from 1959 until 1963. Due to my experience as a patent examiner, I was admitted to practice before the United States Patent & Trademark Office by approximately 1964. During my time as a patent examiner, I was responsible for examining patent applications for small molecule pharmaceuticals and reviewed at least 150 United States patent applications. I received from the Department of Commerce three awards for superior performance during my four-year tenure as a patent examiner.

14. From 1963 until 1971, I was in private practice with Darby & Darby in New York, NY. During my years in private practice, I gained experience in nearly all areas of patent and trademark law, including patent and trademark procurement, opinion writing, client counseling, and intellectual property litigation. During that time, I drafted at least 50 United States patent applications in the chemical arts.

15. In 1971, I joined American Home Products Corporation, which is now known as Wyeth, as a senior patent attorney. In that capacity, I was responsible for filing patent applications, preparing and reviewing intellectual property agreements, and

coordinating with both in-house and outside intellectual property litigation and patent counsel. As a senior patent attorney I drafted at least 40 United States patent applications.

16. In 1974, I was promoted to patent and trademark litigation counsel. My expanded responsibilities included the supervision of company-wide patent and trademark litigation, intellectual property due diligence strategies, and the preparation and review of intellectual property agreements.

17. From 1987 to 2004, I served first as an Assistant General Counsel, and then later as an Associate General Counsel. In 1996, I was promoted to the title of Vice President and Associate General Counsel, Intellectual Property. During this 17-year period, I was responsible for Wyeth's extensive worldwide patent and trademark portfolio, which today includes approximately 2,000 United States patents. As Associate and Assistant General Counsel, I counseled senior management daily regarding intellectual property issues, performed patent infringement and validity analysis, supervised intellectual property litigation, and managed over 30 intellectual property attorneys and agents.

18. I retired from Wyeth on February 1, 2004, and I have consulted for Wyeth and other pharmaceutical companies, including GSK, on a part-time basis since my retirement and continue to do so.

19. I have been a member of professional associations, including the American Bar Association, the Licensing Executives Society, and the American Intellectual Property Association. While employed by Wyeth and since 1987, I

represented the corporation on the Patent Committee of the Pharmaceutical Research and Manufacturers of America (PhRMA), and I was a member of Interpat, an organization composed of chief in-house patent counsel of multinational companies engaged in the research and development of pharmaceutical products.

20. In the past, I have lectured on the interaction of in-house and outside counsel in patent litigation from the perspective of in-house counsel before the American Bar Association, the Practicing Law Institute, the Southwestern Legal Foundation, the New Jersey Patent Law Association, the New York Patent, Trademark and Copyright Association, and at the BNA Annual Patent Conference.

BACKGROUND

21. For the purposes of this report, I have been asked to assume that the facts recited below concerning the circumstances under which the claimed inventions were made are true:

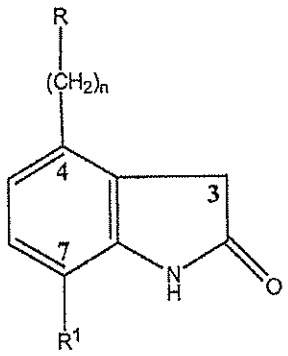
(a) The drug at issue in this case is REQUIP®, which has been used to treat patients suffering from Parkinson's Disease and Restless Legs Syndrome. REQUIP is the commercial name for ropinirole hydrochloride. Two patents relating to this compound are at issue in this litigation. The first, United States Patent No. 4,452,808 (the "'808 patent"), covers the compound itself. The second, United States Patent No. 4,824,860 (the "'860 patent"), covers the use of the compound for the treatment of Parkinson's Disease.

(b) Mr. Gregory Gallagher, a chemist employed at GSK, first synthesized ropinirole. At the time of the invention, Mr. Gallagher was experimenting with a compound described in GSK's U.S. Patent No. 4,314,944 (the "'944 patent") and given the internal designation SK&F 89124 by GSK. Mr. Gallagher synthesized the compound ropinirole by removing the hydroxy (i.e., an oxygen and hydrogen) from the SK&F 89124 compound. Mr. Gallagher sent ropinirole to the GSK pharmacology department to perform routine screening tests to determine whether the compound had cardiovascular utility. Testing by the pharmacology department revealed that ropinirole was a dopamine agonist and exhibited cardiovascular activity. The GSK patent department filed the patent application for ropinirole on December 7, 1982, and a patent was issued on June 5, 1984.

(c) In approximately 1985, responsibility for developing ropinirole was transferred from the GSK Philadelphia offices to the Welwyn offices in the United Kingdom. In the course of performing tests designed to assess the cardiovascular activity of ropinirole, a GSK technician observed stereotypic behavior in rats that had been dosed with ropinirole. This observation led to the discovery by Dr. David Owen, the director of the pharmacology department at Welwyn, that ropinirole could be used for the treatment of Parkinson's Disease. Dr. Owen then retained Professors Brenda Costall and R.J. Naylor of the University of Bradford to perform testing to confirm Dr. Owen's discovery. After Bradford University confirmed Dr. Owen's discovery, Dr. Owen deferred to the GSK patent department to determine the proper scope of the patent application addressing ropinirole's anti-Parkinson's effect. A U.K. patent application was

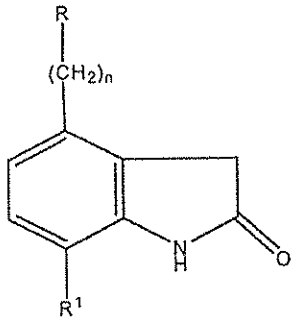
filed on May 21, 1987. The United States patent application, which claims priority to the U.K. application, was filed on May 19, 1988, and the '860 patent issued on April 25, 1989.

22. The '944 patent names William Huffman and James Wilson as inventors and discloses a new group of 2(3H)-indolones whose structures are characterized by a 2(3H)-indolone (oxindole) nucleus having an aminoalkyl substituent at the 4-position and an oxygen function at the 7-position as shown in the generic structure below:



The '944 patent describes two different methods, labeled as Scheme A and Scheme B, by which to synthesize the 2(3H)-indolone compounds disclosed in the patent. The compounds of the '944 patent are described as having beneficial cardiovascular effects and supporting pharmacological data is disclosed for a number of the claimed compounds. The '944 patent also contains six examples. Examples 1, 2, 5, and 6 teach methods of preparing 7-methoxylated 2(3H)-indolones having varying substituents at the 4-position while examples 3 through 6 teach methods of preparing 7-hydroxylated 2(3H)-indolones having varying substituents at the 4-position. Example 1 is a working example while example 6 is a prophetic example. Examples 2 through 5 are combinations of working examples and prophetic examples. Claim 1 of the '944 patent is recited below:

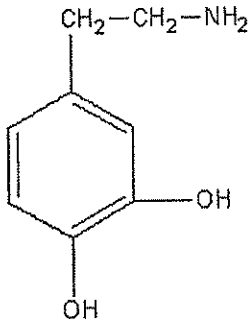
A compound of the structural formula:



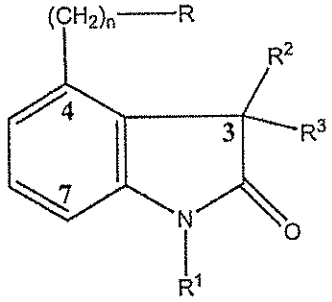
in which R is amino, lower alkylamino, di-loweralkylamino, di-N-allylamino or N-allyl-N-lower alkylamino, R^1 is hydroxy or methoxy and n is an integer from 1-3; together with the pharmaceutically acceptable acid addition salts thereof.

Claims 2 through 9 are directed to compounds with varying substituents at the R and R^1 positions.

23. The '808 patent names Gregory Gallagher, Jr. as the inventor and discloses certain novel 4-aminoalkyl-2(3H)-indolones, which primarily differ from the compounds disclosed in the '944 patent in that they lack a hydroxyl group (or a methoxy group) at the 7-position. The '808 patent describes how the disclosed compounds are dopamine agonists despite the lack of the supposedly essential 7-hydroxy group, a substituent on dopamine which has the structure shown below:

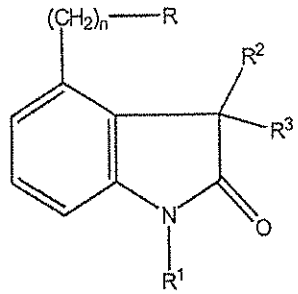


According to the '808 patent disclosure, this 7-hydroxy group was believed necessary for the '944 compounds to resemble the structure of dopamine. The generic chemical structure of the compounds disclosed in the '808 patent is shown below:



The '808 patent describes two different methods, labeled as Scheme A and Scheme B, by which to synthesize the 4-aminoalkyl-2(3H)-indolone compounds disclosed in the '808 patent. The compounds of the '808 patent are disclosed as being peripheral dopamine agonists with beneficial cardiovascular activity as evidenced by supporting pharmacological data for ropinirole hydrochloride, one of the claimed compounds. The '808 patent contains nine examples. Example 1 teaches how to make 4-(2-di-n-propylaminoethyl)-2(3H)-indolone hydrochloride ("ropinirole hydrochloride") by applying Scheme A while example 2 teaches how to make ropinirole hydrochloride by applying Scheme B. Examples 3 through 8 teach methods of preparing compounds of the patent with various 4-position substituents. Example 8 also teaches methods of preparing compounds with 3-position substituents. Example 9 teaches how to make a ropinirole formulation for administration to patient. Examples 1 and 2 are working examples while examples 3 through 7 and 9 are prophetic examples. Example 8 is a combination of a working example and a prophetic example. Claim 1 of the '808 patent is recited below:

A compound of the structural formula:

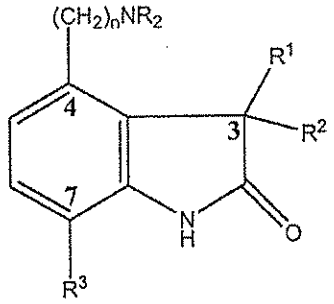


in which: n is 1-3, R is amino, C_{1-6}-lower alkylamino, di-(C_{1-6}-lower alkyl)amino, allylamino, diallylamino, N-(C_{1-6}-lower alkyl)-N-allylamino, benzylamino, dibenzylamino, phenethylamino, diphenethylamino, 4-hydroxyphenethyl amino or di-(4-hydroxyphenethyl)amino, and R^1, R^2 and R^3 are, each, hydrogen or C_{1-4}-lower alkyl; or a pharmaceutically acceptable, acid addition salt thereof.

Claims 2 through 7 are directed to compounds with varying substituents at the R, R^1, R^2, and R^3 positions. Claim 8 is directed to a pharmaceutical composition having D_2 receptor agonist activity comprising a nontoxic, agonist quantity of a compound of the structural formula shown above for claim 1, in dosage unit form, combined with a pharmaceutical carrier. Claims 9 and 10 are directed to the composition of claim 8 in which the D_2-agonist compound is 4-(2-di-n-propylaminoethyl)-2(3H)-indolone or 4-(2-di-n-propylaminoethyl)-2(3H)-indolone hydrochloride, respectively, while claim 11 is directed to the composition of claim 8 in dosage unit form adapted for use as an antihypertensive composition. Claim 12 is directed to a specific quantity per unit dosage.

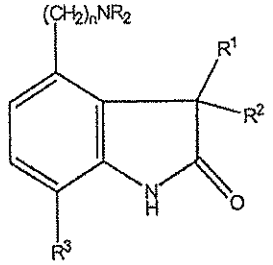
24. The '860 patent lists David Owen as the inventor and claims a method of treatment of Parkinson's Disease by the administration of certain indolone derivatives. The indolone derivatives disclosed in the '860 patent include some of the compounds disclosed in the '944 and '808 patents. The '860 patent discloses the discovery that these

prior art indolone compounds, previously thought to be peripheral dopamine agonists, exhibit central nervous system ("CNS") effects and could be a successful treatment for Parkinson's Disease. The generic chemical structure of the compounds disclosed in the '860 patent is shown below:



There are seven examples (A-G) in the '860 patent. Example A describes the effect of ropinirole hydrochloride, one of the claimed compounds, on spontaneous locomotor activity in mice. The results of this test indicate that ropinirole hydrochloride has dopamine agonist activity. Example B describes the ability of ropinirole hydrochloride to induce stereotypy in rats or mice. The results of this test are indicative of a more selective mode of dopamine agonist action. Example C demonstrates that ropinirole hydrochloride has anti-Parkinson potential based on its effect on locomotor activity in the rat. Example D shows that ropinirole hydrochloride exhibits statistically significant anti-depressant activity based on its effect in the Porsolt Test. Example E demonstrates ropinirole hydrochloride's anxiolytic effects and example F demonstrates the anti-Parkinson activity of ropinirole hydrochloride in the MPTP-Treated Marmoset Model. Example G shows the results of receptor binding studies, demonstrating that ropinirole hydrochloride is more selective in its binding to dopamine receptors than other D_2

agonists, bromocriptine and pergolide. All of the examples in the '860 patent are working examples. Claim 1 of the '860 patent is recited below:

> A method of treatment of Parkinsons Disease which comprises administering an effective non-toxic amount for the treatment of Parkinsons Disease of a compound of the following structure:
>
> 
>
>
> in which each group R is hydrogen or C_{1-4} alkyl;
> R^1 and R^2 are each hydrogen or C_{1-4} alkyl;
> R_3 is hydrogen or hydroxy; and
> n is 1 to 3;
> or a pharmaceutically acceptable salt thereof to a subject in need thereof.

Claims 2 and 3 are directed to a method of treatment of Parkinsons Disease which comprises administering an effective non-toxic amount for the treatment of Parkinsons Disease of 4-(2-di-n-propylaminoethyl)-2-(3H)-indolone or 4-(2-di-n-propylaminoethyl)-2-(3H)-indolone hydrochloride, respectively, to a subject in need thereof.

OPINIONS

I. Teva's Allegations of Inequitable Conduct with Respect to the Generic Claims of the '808 and '860 Patents.

A. The Generic Claims of the '808 Patent

25. Teva has alleged that the '808 patent is invalid because, in addition to ropinirole hydrochloride which is claimed in dependent claim 5, the patent covers several

related compounds in claims 1-4, 6-9, and 11-12, which Mr. Gallagher allegedly did not invent. See Teva Corrected and Amended Counterclaim ¶¶ 34-43. According to Teva, "the applicants' nonjoinder of individual(s) responsible for conceiving of portions of the claimed invention(s) covering compounds other than ropinirole or its hydrochloride salt and Mr. Gallagher's and the applicants' submission of the false declaration of inventorship were done with deceptive intent." Corrected and Amended Counterclaim ¶ 43.

26. Teva's theory of inequitable conduct reflects a fundamental misunderstanding of the claim drafting process and the role of the patent practitioner before the United States Patent & Trademark Office in attempting to obtain patent protection for an invention relating to a pharmaceutical compound. If accepted generally, it would substantially and retroactively curtail the scope of protection available to companies for new and useful compounds and could render numerous patents, including those of Teva, unenforceable.

27. Inventors in a pharmaceutical company, as elsewhere, have varying degrees of familiarity with the patent prosecution process. Inventors are very rarely lawyers or patent agents and therefore naturally rely on the advice and judgment of the lawyers or patent agents responsible for obtaining patent protection for the invention. Among the duties of the patent practitioners before the United States Patent & Trademark Office is the obligation to obtain the broadest claims possible that meet the requirements of Title 35, consistent with the disclosure of the patent application, the scope of the prior art of which he or she is aware, and the duty of candor. Accordingly, prosecuting attorneys are trained to draft and seek to obtain, broad, generic claims, which fairly

reflect the contribution of the inventor to the art. In doing so, they routinely seek and are granted protection beyond the specific embodiment(s) discovered or developed by the inventor. Treatises and written guidance addressing patent drafting confirm that this is the approach that should be followed in drafting genus claims.[1/]

28. With respect to chemical patents in particular, a patent limited to the precise compounds reduced to practice by an inventor would frequently be of little or no value because of the ability to obtain the same functionality of the compound by making minor substituent variations or manipulations to the molecule. Thus, it is normal practice for a patent attorney to draft a patent application to include generic formulas that would include any related substituents that could reasonably be expected to exhibit similar activity and to seek claims to such generic formulas. This does not make the inventor of the chemical compound any less the inventor of the generic formula. Rather, invention of the specific compound entitles the inventor to a genus of compounds of reasonable scope.

[1/] See, e.g., Robert C. Faber, *Landis on Mechanics of Claim Drafting*. § 10:1.1, 5th Ed., 2005 ("Broad coverage means not only that every particular preferred disclosed embodiment is protected in the claims, but that the claims cover all expected and unanticipated equivalents that competitors and others may later develop and all intentional and unintentional copies of the claimed invention which embody the inventor's concept. The inventor/client will compare a competitive or a similarly functioning product or process with the patented embodiments. If the client sees similar structure, operation and/or result, he will want to be able to use his patent to halt an infringement. It is the claim drafter's job to have written the claims in the application to not only cover what the attorney and the inventor/client could at the time of application prosecution have envisioned as competing products, but to cover competitive products which neither the inventor nor the attorney thought of or could even have imagined at the time, but which employ the concept of the invention."); Jeffrey G. Sheldon, *How to Write a Patent Application*, Practicing Law Institute, § 6.5, 2006 ("The broadest claim should be as broad as possible in view of the prior art. As long as the broad claim is not anticipated by art known to the inventor, it cannot hurt to ask for the broad claim. At worst, the examiner will not allow the broadest claims. Thus, it is recommended that the practitioner be greedy when initially writing the application."); Irving Kayton, *Kayton on Patents*, 2nd ed., 3-1, 1983 ("During the prosecution stage the drafter will naturally attempt to write one claim that is as broad as the prior art of which he is aware will permit and that is supported by the disclosure in his patent application.").

29. A genus or generic claim is one that covers more than a single chemical compound. The practice of obtaining generic claims described above is consistent with standard industry practice and basic patent law principles, as I understand them. It is typical for a patent attorney or agent to more broadly claim the invention to include other species that are envisioned to have the same utility and can be similarly made in order to ensure that the invention is protected. This broadened concept becomes the genus in a patent application. Moreover, the existence of only one working example or even the possibility of inoperative species does not automatically result in the genus claim not meeting the statutory requirements. It is acceptable for a specification to merely contain a written description of the broadly claimed invention without having to, in addition, detail every species that is encompassed by such a genus claim.[2/]

30. Based on the facts set forth above and the facts which I have been asked to assume as true, the prosecution of the '808 patent appears to have been entirely consistent with normal patent prosecution practice related to the prosecution of generic chemical claims. Mr. Gallagher was the first and only person to initially synthesize ropinirole, and

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[2/] These standard industry practices have been upheld by the courts as permissible as a matter of law. See, e.g., *In re Fisher*, 427 F.2d 833, 839 (C.C.P.A. 1970) (stating that as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied); *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 974 (Fed. Cir. 2002) (Lourie, J., concurring in decision to deny rehearing en banc) ("Although one may envision a general concept, what one usually does first in making or isolating a chemical or chemical-related invention is to obtain a specific material or materials. One then broadens the concept to extend it as far as one envisions that other materials will have the same utility and can be similarly made. That broadened concept becomes the genus in a patent application that is both the broadest statement constituting a written description and usually claim 1."); *Atlas Powder Co. v. E.I. du Pont de Nemours and Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984) (holding that the presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled); *Utter v. Hiraga*, 845 F.2d 993, 998 (Fed. Cir. 1988) ("A specification may, within the meaning of 35 U.S.C. §112 ¶ 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses.").

it was he who was responsible for sending the compound to GSK's pharmacology department for routine testing as to its utility. Mr. Gallagher apparently did not make any other compounds coming within the scope of claim 1 of the '808 patent, but the application for the '808 patent does not purport to describe any working examples of other compounds. Even assuming Mr. Gallagher did not make such compounds, Teva's counterclaim alleging improper inventorship fails to identify by name any alleged co-inventors.

31. As discussed above, the specification of the '808 patent describes a way to synthesize ropinirole and provides data supporting its utility. The '808 patent also provides several prophetic examples of the synthesis of related compounds other than ropinirole coming within the generic formula. In light of the disclosure of the '808 patent and the disclosure of the '944 patent (which is cited in the '808 patent as prior art), the generic claim sought by the applicant in the '808 patent is most reasonable, and it is not surprising that the examiner allowed it. No objection was made by the examiner that any species within the genus claim of the '808 patent did not have utility. This is consistent with the Manual of Patent Examining Procedure's ("MPEP")[3/] guidance for patent examiners when considering the scope of generic claims:

> With respect to the adequacy of disclosure that a claimed genus possesses an asserted utility representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if it would be deemed likely by one skilled in the art, in view of contemporary knowledge in the art, that the claimed genus would possess the asserted utility. Proof of utility will be required for other members of the claimed genus only in those cases where

[3/] The MPEP is published by the USPTO to provide patent examiners, applicants, attorneys, and those involved in the prosecution of a patent with instruction and reference on the patent prosecution practices and procedures before the USPTO.

> adequate reasons can be advanced by the examiner for believing that the genus as a whole does not possess the asserted utility.

MPEP § 608.01(p) at pp. 102-3 (4th ed., rev. Sep. 1982) (citations omitted).[4/] The patent examiner who examined the '808 patent had before him the genus disclosed in the '944 patent, which had been cited to the PTO by the applicant and which was the closest prior art. Given the disclosure, it would have been reasonable to conclude that the claimed genus of the '808 patent would possess the asserted utility.

32. In considering the issues in this case, I have reviewed several Teva patents to compare them to the '808 patent. Not surprisingly, it appears that Teva has commonly sought and obtained broad generic claims notwithstanding a patent disclosure that describes a relatively small number of closely related species actually made and tested by the inventors relative to the scope of the broadest generic claim. See Attachment C. For example, claims 1 and 7 of Teva's U.S. Patent No. 5,585,358 ("'358 patent"), directed towards derivatives of 2-propylpentanoic acid (valproic acid) and 2-propyl-2-pentenoic acid, have broader genus claims than the '808 patent claims. Claim 1 describes a compound with substituents R_1, R_2, and R_3 "wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1-C_6 alkyl group, an aralkyl group, or an aryl group, and n is equal to 0," while Claim 7 recites a structure "wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1-C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3." The '358 patent describes a broad and varied list of aralkyl and aryl

[4/] The above quote is from the September 1982 revision of the MPEP, which was in effect at the time of the filing of the '808 patent in December 1982. There was another MPEP revision in August 1983, which recites the same language as quoted above. In addition, the MPEP's guidance on the utility of genus claims did not substantively change in its 1988 through 1989 editions which were in effect at the time of the filing and prosecution of the '860 patent.

substituents in column 2, lines 60-68, and in column 3, lines 1-2, with only two working examples to illustrate those broad classes of structurally diverse substituents. Only a single working example appears in the '358 patent to support at least 15 diverse substituents represented by "R_1, R_2, R_3" which are recited in claim 5 of this patent.

33. By way of further example of standard industry practice, the patent underlying the world's best selling drug, Pfizer's Lipitor®, claims a genus that is greater in size and scope than that claimed in the '808 patent. Claim 1 of U.S. Patent No. 4,681,893 (the "'893 patent") designates a wide-ranging scope of possible substituents at positions R_1-R_4, yet only provides four exemplified species. See Attachment D. The patent claims remain valid today, nearly twenty years after issue, despite challenges by generic manufacturers. Most recently, the '893 patent was upheld in *Pfizer, Inc. v. Ranbaxy Labs.*, 06-1179 (Fed. Cir. August 2, 2006), appealed from *Pfizer, Inc. v. Ranbaxy Labs.*, 405 F. Supp. 2d 495 (D. Del. 2005).[5/]

34. To summarize, the prosecution of the '808 patent is consistent with the practice of prosecuting pharmaceutical patents that has been commonplace throughout my career. A patent application was drafted which reflects Mr. Gallagher's actual work and includes prophetic examples, the accuracy of which Teva does not contest.

B. The Generic Claims of the '860 Patent

35. It is also my understanding that Teva attacks the generic claims contained in the '860 patent on similar grounds. Teva asserts that claim 1 of the '860 patent claims indolone compounds other than simply ropinirole or its hydrochloride salt. According to

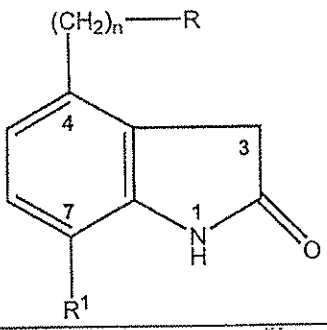
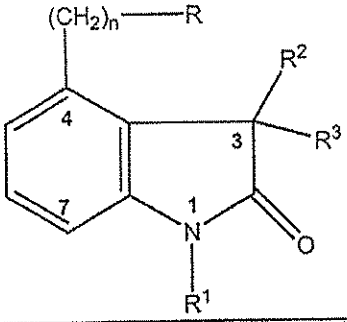
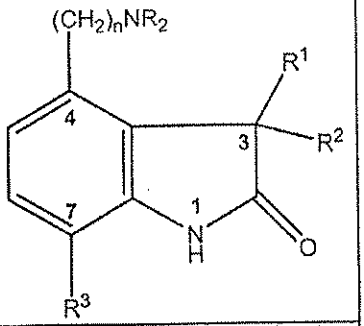
[5/] In fact, based upon both opinions, it does not appear that the validity of the generic claim was even challenged in the lawsuit.

Teva, "[p]laintiffs' nonjoinder of individual(s) responsible for conceiving of portions of the claimed inventions(s) covering compounds other than ropinirole or its hydrochloride salt was done with the intent to deceive the PTO so that the '860 patent would be issued." Corrected Amended Complaint ¶ 60.

36. The '860 patent does not suggest that any compound other than ropinirole had been actually tested for its anti-Parkinson's Disease effect. As with the '808 patent, GSK was nonetheless entitled to a genus claim of appropriate scope. The generic claim of the '860 patent that was sought and obtained by GSK is reasonable in light of the disclosure of the '860 patent and the prior art.

37. Given the teaching of the '944 and '808 patents, it would have been reasonable to seek a genus claim in the '860 patent of substantially the same scope as the '808 patent and the '944 patent. However, the genus in claim 1 of the '860 patent is narrower at the 4-position than the genus claimed in the '944 or '808 patents as shown in the chart below comparing the genus claims of these patents. The genus of claim 1 of the '860 patent is also narrower than the genus of the '808 patent at the 1-position as shown below. In the '860 patent, hydrogen is bound to the indole nitrogen ring, whereas in the '808 patent there may be either hydrogen or a lower-alkyl substituent bound to the indole nitrogen ring.

COMPARISON OF GENUS CLAIMS IN '944, '808, & '860 PATENTS

'944 Patent	'808 Patent	'860 Patent
		
R	**R**	**NR_2**
Amino	Amino	Amino (when both **R**'s = H)
lower alkylamino	C_{1-6} lower alkylamino	C_{1-4} alkylamino (when one **R** = H and the other **R** = C_{1-4}-lower alkyl)
di-loweralkylamino	di-(C_{1-6}-loweralkyl)amino	di-(C_{1-4}-loweralkyl)amino (when both **R**'s = C_{1-4}-lower alkyl)
	allylamino	
di-N-allylamino	diallylamino	
N-allyl-N-lower alkylamino	N-(C_{1-6}-lower alkyl)-N-allylamino	
	benzylamino, dibenzylamino	
	phenethylamino, diphenethylamino	
	4-hydroxyphenethyl amino, di-(4-hydroxyphenethyl)amino	
R^1	**(position not designated)**	**R^3**
Hydroxy (OH)		Hydroxy
Methoxy (OCH_3)		
	Hydrogen	Hydrogen
(position not designated)	**R^1**	**(position not designated)**
Hydrogen	Hydrogen	Hydrogen
	C_{1-4}-lower alkyl	
(position not designated)	**R^2, R^3 (independently)**	**R^1, R^2 (independently)**
Hydrogen	Hydrogen	Hydrogen
	C_{1-4}-lower alkyl	C_{1-4}-lower alkyl
n	**n**	**n**
1-3	1-3	1-3

The fact that GSK drafted narrow genus claims appropriate for each patent reflects a careful and considered approach to prosecuting this family of patents.

38. As with the '808 patent, the prosecution of the genus claim in the '860 patent proceeded in a manner consistent with standard practice in the pharmaceutical industry then and today. Dr. Owen appropriately relied on his patent attorney to obtain a claim of appropriate scope in light of the prior art and the disclosure of the '860 patent. The claim sought by GSK is reasonable but, in any event, it is not inequitable conduct to seek claims as broad as possible taking into account the teachings of the specification and the prior art.

II. The Use of Prophetic Examples Regarding Human Dosing

39. It is my understanding that Teva alleges that the '808 patent is invalid because it asserts that GSK misled the Patent Office into believing that it had already tested for an effective dose in humans. This allegation is unfounded in light of the actual disclosure of the '808 patent.

40. Pharmaceutical patents sometimes include a human dose recommendation. The '808 patent is no different. It states:

> Advantageously, doses selected from the dosage unit ranges given above will be administered several times, such as from one to five times, a day. The daily dosage regimen is selected from the range of about 50 mg to about 1.0 mg, preferably 200-750 mg for oral administration and 50-500 mg for parental administration. When the method described above is carried out, D2-agonist activity is produced.

> For an average size human using 4(2-di-n-propylaminoethyl)-2(3H)-indolone hydrochloride as an active ingredient, a typical dose to show anti-hypertensive activity would be selected from the range of from about 100-250 mg of base equivalent for each dosage unit which is adapted for oral administration and which is administered orally from 1-4 times daily.

Column 5, line 59 to col. 6, line 5.

41. Based on my experience as a patent attorney and patent examiner, it is my opinion that the above excerpt is a prophetic example. In the drafting of patents, the distinction between actual "working" examples and "prophetic" examples is well established. According to the MPEP, working examples correspond to work actually performed. MPEP § 608.01(p) at 104. However, prophetic examples are also common in patents:

> Simulated or predicted test results and prophetical examples (paper examples) are permitted in patent applications. . . Paper examples describe the manner and process of making an embodiment of the invention which has not actually been conducted.

Id. Working examples typically use the past tense to describe the actual work performed, while "[p]aper examples should not be described using the past tense." *Id.*[6] Therefore, because of the absence of the use of the past tense throughout the dosing discussion of the '860 patent, a patent examiner would not have interpreted this dosage recommendation as a representation of testing that had already been completed. Indeed, this passage actually employs the future tense as well as the present tense, emphasizing the predictive intent of the statements, including, for example, "doses selected . . . *will be*

[6] The August 1983 MPEP revision recites the same language with regard to prophetic examples as the September 1982 edition quoted above.

administered[,]" "*[w]hen* the method described above is carried out ...[,]" and "a typical dose to show anti-hypertensive activity *would be* selected . . . ." The future tense is clearly utilized to signal to the patent examiner that this is a prophetic example. It is common for pharmaceutical patents to have prophetic examples, particularly when it comes to dosing recommendations. I note that Teva appears to have employed a prophetic example with regard to the effectiveness of a claimed dose in one of its own pharmaceutical patents.[7/]

42. In addition, as a matter of common sense, a new chemical compound that is the subject of a pharmaceutical patent application would often and most likely not have been actually tested in humans at the time the application was filed. The Hatch-Waxman Act -- the federal statute that is the basis for ANDA filings such as the Teva ANDA at issue in this proceeding -- also provides for patent term extension precisely because of the loss of patent life due to FDA-required human clinical testing. Yet, a pharmaceutical company must file its patent application at an early stage or run the risk that others may publish on the company's compounds and impair future patent rights abroad where jurisdictions follow "a first to file" system. In fact, a patent examiner would understand that the actual results of human testing would typically be available only for a second or third generation improvement for which patent protection is sought to an existing patented compound.[8/]

[7/] For example, see Teva U.S. Patent No. 6,569,459 directed to a method of administration of paclitaxel-plasma protein formulation. See Attachment E. Example 2 of the '459 patent is written in the present tense as contrasted with Example 1,and suggests a protocol for evaluating the claimed method using a mouse experimental model. The example even goes so far as to generate a table listing experimental values that might be expected to result from such an experiment.

[8/] For examples of Teva patents with claims broadly directed toward therapeutic treatment of a "patient" or a "subject" but disclosing only testing in mice and rats, see U.S. Patent Nos. 6,569,459 (see footnote 7) and 5,585,358 (discussed *supra*).

III. TRIAL EXHIBITS

43. I may rely on visual aids and demonstrative exhibits that demonstrate the basis for my opinion. Examples of these visual aids and demonstrative exhibits may include, for example, diagrams of one or more of the compounds claimed, and blow-ups of documents considered or excerpts from this report.

IV. RESERVATION OF RIGHTS

44. My opinions are based upon the information that I have considered to date. I reserve the right to supplement or amend my opinions in response to opinions (including any rebuttal opinions) expressed by Teva's experts by or in light of any additional evidence, testimony, or other information that may be provided to me after the date of this report, including at trial.

Date: September 22, 2006

Egon E. Berg

Egon E. Berg

ATTACHMENT A

EGON E. BERG
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(201) 652-0624; (201) 652-9221 fax
E-mail: bezyberg@hotmail.com

PROFESSIONAL EXPERIENCE

1971 – 2004 American Home Products Corporation/Wyeth (Fortune 100 Company)

1996-2004 **Vice President and Associate General Counsel**

1992-1996 **Associate General Counsel**

1987-1992 **Assistant General Counsel**

Responsible for company-wide IP, including extensive worldwide patent and trademark portfolio; daily senior management counseling, infringement and validity analysis; legal review of IP license agreements, IP due diligence strategies and reviews for the acquisition of businesses and products; supervision of IP litigation, including tactics, strategy; management of 80+ IP attorney and administrative support team.

1974-1987 **Litigation Counsel, Patents and Trademarks**

Responsible for corporate-wide patent and trademark litigation; coordination with and supervision of outside counsel; review of legal briefs, strategy for litigation; direct handling of trademark litigation; IP due diligence strategies and reviews for the acquisition of businesses and products; preparation and review of IP agreements.

1971-1974 **Senior Patent Attorney**

Patent application filing and procurement; preparation and review of IP agreements; supervision of and coordination with outside counsel of IP litigation.

1963-1971 **Darby & Darby** (New York City)
Extensive practice in all phases of patent and trademark law, including patent and trademark procurement, opinion writing, client counseling and IP litigation.

1959-1963 **United States Patent & Trademark Office**
Pharmaceutical Group 120, Patent Examiner

BAR ADMISSIONS

New York, District of Columbia, U.S. Supreme Court and CAFC

EDUCATION

BS Chemistry – 1959 Rutgers University
JD - 1963 George Washington University

OTHER PROFESSIONAL ACTIVITIES

Lecturer PLI, Southwest Legal Foundation

ATTACHMENT B

DOCUMENTS CONSIDERED BY EGON E. BERG

GSK-REQ011545-GSK-REQ019834

GSK-REQ024778-GSK-REQ024780

GSK-REQ00674-GSK-REQ006749

GSK-REQ013314- GSK-REQ013313

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GSK-REQ094317-GSK-REQ094863

Deposition Transcript of David Owen, without exhibits, May 26, 2006

Deposition Transcript of Gregory Gallagher, Jr., without exhibits, May 5, 2006

Teva's Motion for Leave to Amend Its Answers, Defenses, and Counterclaims, with exhibits, June 28, 2006

Teva's Third Supplemental Response to Plaintiff's First Interrogatories, June 9, 2006

Expert Report of Dr. Joseph G. Cannon, with attachments, July 10, 2006

Expert Report of Dr. John Paul Long, with attachments, July 10, 2006

Expert Report of Dr. Daniel Tarsy, with attachments, July 10, 2006

Plaintiff's Brief in Opposition to Defendant's Motion for Leave to Amend its Answer, Defenses and Counterclaims, with exhibits, July 13, 2006

Defendant Teva's Corrected Brief In Support of Motion for Leave to Amend its Answer Defenses, and Counterclaims, July 10, 2006

Defendant Teva's Corrected Motion for Leave to amend its Answer, Defenses, and Counterclaims, July 10, 2006

Defendant's Reply Brief in Support of Motion for Leave to Amend its Answer, Defenses and Counterclaims, with exhibits, July 24, 2006

United States Patent No. 6,569,459

United States Patent No. 5,585,358

United States Patent No.4,681,893

United States Patent No.5,576,353

European Patent No. 0113964-B1

European Patent No. 0299602-B1

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Sheldon, Jeffrey G., *How to Write a Patent Application*, Practicing Law Institute, § 6.5, 2006

Kayton, Irving, *Kayton on Patents*, 2nd ed., 3-1, 1983

Allen Organ Co. v. Kimball Int'l, Inc., 839 F.2d 1556 (Fed. Cir. 1988)

In re Angstadt, 537 F.2d 498 (CCPA 1976).

Atlas Powder Co. v. E.I. Du Pont de Nemours and Co., 750 F.2d 1569 (Fed. Cir. 1984)

Board of Education v. American Bioscience, Inc., 333 F. 3d 1330 (Fed. Cir. 2003)

In re Borkowski et al., 422 F.2d 904 (CCPA 1970)

Bristol-Myers Squibb Co. v. Rhone-Poulenc Rorer, Inc., 326 F.3d 1226 (Fed. Cir. 2003)

Bruno Indep. Living Aids, Inc. v. Acorn Mobility Servs., Ltd., 394 F.3d 1348 (Fed. Cir. 2005)

Burlington Indus., Inc. v. Dayco Corp., 849 F.2d 1418 (Fed. Cir. 1988)

Burroughs Wellcome Co. v. Barr Labs., Inc., 40 F.3d 1223 (Fed. Cir. 1994).

In re Cavallito et al., 282 F.2d 363, 127 USPQ 202 (CCPA 1960)

Critikon, Inc. v. Becton Dickinson Vascular Access, Inc., 120 F.3d 1253 (Fed. Cir. 1997).

In re Dreshfield, 110 F.2d 235, 45 USPQ 36 (CCPA 1940),

In re Edwards, 568 F.2d 1349, 1351-2 (CCPA 1978)

Enzo Biochem, Inc v. Gen-Probe Inc., 323 F.3d 956 (Fed. Cir. 2002)

Ferring B.V. v. Barr Laboratories, Inc., 437 F.3d 1181 (Fed. Cir. 2006)

Fina Oil & Chemical Co. v. Ewen, , 123 F.3d 1466 (Fed. Cir. 1997)

In re Fisher, 427 F.2d 833 (CCPA 1970)

Ex parte Forman et al., 230 USPQ 546 (BPAI 1986)

Frazier v. Roessel Cine Photo Tech., Inc., 417 F.3d 1230 (Fed. Cir. 2005)

Fujikawa v. Wattanasin, 93 F.3d 1559 (Fed. Cir. 1996)

Genentech, Inc. v. Novo Nordisk, A/S, 108 F.3d 1361 (Fed. Cir. 1997)

GFI, Inc. v. Franklin Corp., 265 F.3d 1268 (Fed. Cir. 2001)

Hess v. Advanced Cardiovascular Sys., Inc., 106 F.3d 976 (Fed. Cir.)

Kingsdown Med. Consultants, Ltd., v. Hollister, Inc, 863 F.2d 867 (Fed. Cir. 1988)

In re Marzocchi, 439 F.2d 220 (CCPA 1971)

Miller v. Walker, 214 USPQ 845 (Bd. Pat. Int.1982).

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Nordberg, Inc. v. Telsmith, Inc., 82 F.3d 394 (Fed. Cir. 1996).

Novo Nordisk Pharms., Inc. v. Bio-Technology General Corp., 424 F.3d 1347 (Fed. Cir. 2005)

Oka v. Youssefyeh, 849 F.2d 581 (Fed. Cir. 1988)

Paragon Podiatry Lab., Inc. v. KLM Labs., Inc., 984 F.2d 1182 (Fed. Cir. 1993).

PerSeptive Biosystems v. Pharmacia Biotech, 225 F.3d 1315 (Fed. Cir. 2000)

Pfizer, Inc v. Ranbaxy Labs, 06-1179 (Fed. Cir. August 2, 2006)

Pfizer, Inc v. Ranbaxy Labs, 405 F. Supp. 2d 495 (D.Del. 2005)

Pharmacia Corp. v. Par Pharmaceutical, Inc., 417 F.3d 1369 (Fed. Cir. 2005)

In re Rainer et al., 377 F.2d 1006 (CCPA 1967)

Refac International, Ltd. v. Lotus Development Corp., 81 F.3d 1576 (Fed. Cir. 1996)

Semiconductor Energy Lab. Co. v. Samsung Elecs. Co., 204 F.3d 1368 (Fed. Cir. 2000)

Sewall v. Walters, 21 F.3d 411 (Fed. Cir. 1994).

Ex parte Sudilovosky 21 PQ.2d 1702 (BPAI 1991)

Regents of the Univ. of California v. Eli Lilly & Co., 119 F.3d 1559 (Fed. Cir. 1997)

In re Ruschig (379 F.2d 990, CCPA 1967)

Utter v. Hiraga, 845 F.2d 993 (Fed. Cir. 1988)

In re Vaeck, 947 F. 2d 488 (Fed. Cir. 1991)

In re Wands, 858 F.2d 731 (Fed. Cir. 1988)

In re Wright, 999 F.2d 1557 (Fed. Cir. 1993)

MPEP § 608.01 (p) 4th ed., rev. 9 Sept. (1982)

MPEP § 608.01 (p) 5th ed., rev Aug. (1983)

MPEP § 608.01 (p) 5th ed., rev. July. (1987)

MPEP § 608.01 (p) 5th ed., rev. Oct. (1987)

MPEP § 608.01 (p) 5th ed., rev. Dec. (1987)

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MPEP § 608.01 (p) 5th ed., rev. 9 Sept. (1988)

MPEP § 608.01 (p) 5th ed., rev. Jan. (1989)

MPEP § 608.01 (p) 5th ed., rev. Apr. (1989)

ATTACHMENT C

US005585358A

United States Patent [19]

Bialer et al.

[11] Patent Number: 5,585,358

[45] Date of Patent: Dec. 17, 1996

[54] DERIVATIVES OF VALPROIC ACID AMIDES AND 2-VALPROENOIC ACID AMIDES, METHOD OF MAKING AND USE THEREOF AS ANTICONVULSANT AGENTS

[75] Inventors: Meir Bialer, Jerusalem; Salim Hadad, Kfar Peki'in; Jacob Herzig, Ra'anana; Jeff Sterling, Jerusalem; David Lerner, Jerusalem; Mitchell Shirvan, Jerusalem, all of Israel

[73] Assignees: Yissum Research Development Corporation of the Hebrew University of Jerusalem; Teva Pharmaceutical Industries Ltd., both of Jerusalem, Israel

[21] Appl. No.: 88,074

[22] Filed: Jul. 6, 1993

[51] Int. Cl.[6] C07K 5/067; A61K 38/05

[52] U.S. Cl. 514/19; 564/155; 564/159

[58] Field of Search 564/155, 159; 514/19

[56] References Cited

U.S. PATENT DOCUMENTS

4,639,468 1/1987 Roncucci et al. .

FOREIGN PATENT DOCUMENTS

0046707 3/1982 European Pat. Off. .
0250997 1/1988 European Pat. Off. .
0442012 8/1991 European Pat. Off. .

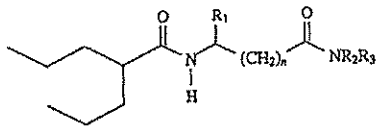
OTHER PUBLICATIONS

Granneman, Xenobiotica 14 (5)375(1984).
Yu, Mol. & Chem Neuropath 15, 37 (1991).
Chemical Abstracts 101 (17), 143458v, 1984.
Drugs of the Future 9(8):587–588 (1984).
Drugs of the Future 16(8):775 (1991).
Haj-Yehia, A., et al., *Pharmaceutical Research* 6(8):683–689.
Bialer, M., et al., *Eur. J. Clin. Pharmacol.* 38:289–291 (1990).
Bialer, M., *Clin. Pharmacokinet.* 20(2):114–122 (1991).
Hadad, S., et al., *Journal of Pharmaceutical Sciences* 81(10):1047–1050 (1992).

Primary Examiner—Robert Gerstl
Attorney, Agent, or Firm—John P. White

[57] ABSTRACT

A compound having the structure:



wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3. Also provided are a compound containing a 2-valproenoic moiety, pharmaceutical compositions comprising these compounds, and methods of using them for the effective treatment of epilepsy and other neurological disorders.

19 Claims, 2 Drawing Sheets

FIGURE 1

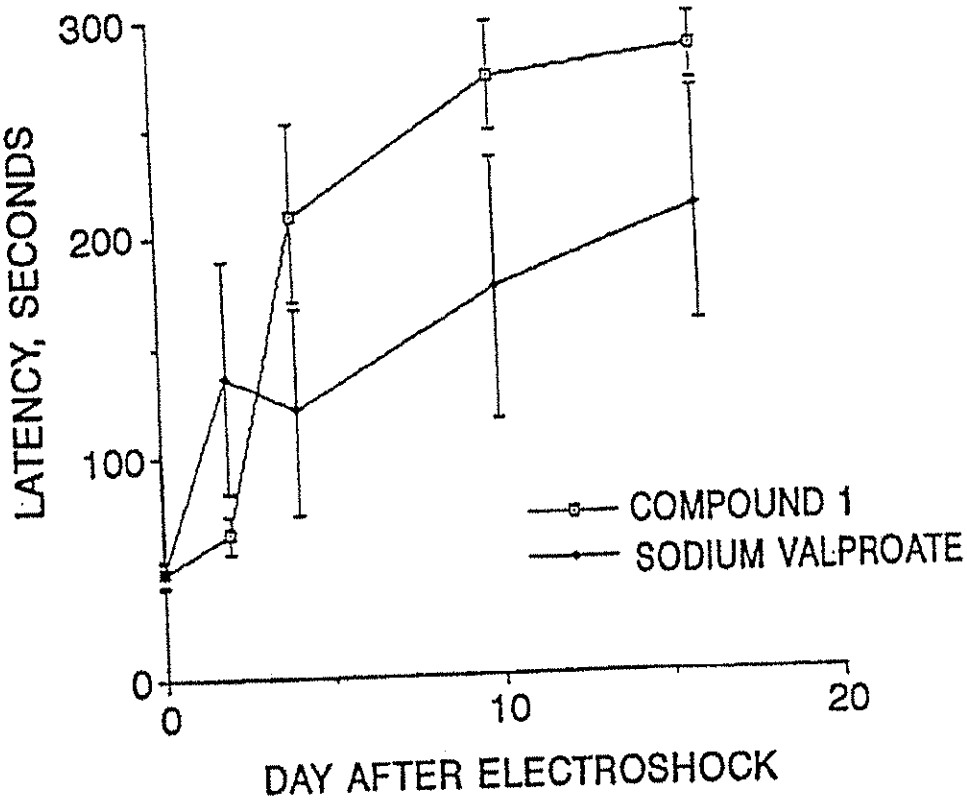
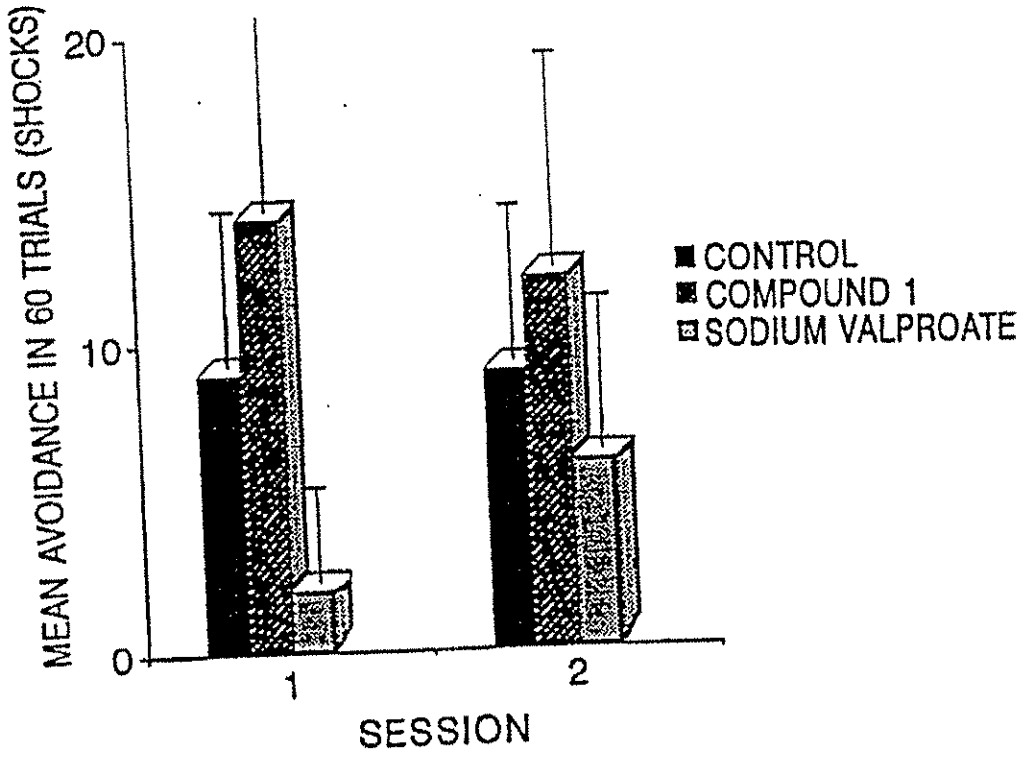

300
200
100
0
LATENCY, SECONDS
0
10
20
DAY AFTER ELECTROSHOCK
COMPOUND 1
SODIUM VALPROATE

FIGURE 2


MEAN AVOIDANCE IN 60 TRIALS (SHOCKS)
20
10
0
1
2
SESSION
CONTROL
COMPOUND 1
SODIUM VALPROATE

DERIVATIVES OF VALPROIC ACID AMIDES AND 2-VALPROENOIC ACID AMIDES, METHOD OF MAKING AND USE THEREOF AS ANTICONVULSANT AGENTS

BACKGROUND OF THE INVENTION

The invention relates to new derivatives of 2-propylpentanoic acid (valproic acid, hereinafter VPA), and 2-propyl-2-pentenoic acid, their preparation and use as antiepileptic agents.

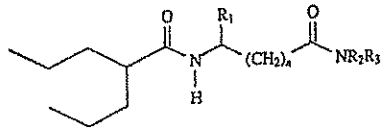
VPA and its alkali salts are major drugs in the arsenal of drugs for the treatment of epileptic seizures and convulsions. However, approximately 25% of epileptic patients do not respond to current treatment. Furthermore, VPA itself has considerable adverse effects including hepatotoxicity and teratogenicity. Baille, T. A. and A. W. Rettenmeier, in "Antiepileptic Drugs," ed. by R. H. Levy, F. E. Dreifuss, R. H. Mattson, B. S. Meldrum and J. K. Penry, Raven Press, New York (1989), at 601–619.

One approach to obtain improved antiepileptic agents has been to prepare the primary amide derivatives of VPA and its analogs. M. Bialer, *Clin. Pharmacokinet.* 20:114–122 (1991); M. Bialer, A. Haj-Yehia, N. Barzaghi, F. Pisani, and E. Perucca, *Eur. J. Clin. Pharmacol.*, 289–291 (1990); A. Haj-Yehia and M. Bialer, *J. Pharm. Sci.*, 79: 719–724 (1990). While certain glycinamide derivatives have been disclosed by R. Roncucci, et al., U.S. Pat. No. 4,639,468, issued Jan. 27, 1987, these compounds generally have not been accepted into clinical practice. Thus, an urgent need still exists in the art for developing anti-convulsant agents with improved efficacy and a wider margin between the dose which is therapeutic and that which is neurotoxic.

VPA and 2-ene-VPA-related glycine amides have been disclosed by Granneman, et al., *Xenobiotica*, 14, 375 (1984), to be minor metabolites of VPA. However, an examination of the mass spectral data therein shows that those compounds are in fact VPA and 2-ene-VPA glycine and cannot be glycinamide conjugates, wherein the glycine nitrogen moiety is attached to the VPA or 2-ene-VPA carbonyl. While Granneman, et al., described these compounds as glycine conjugates, they erroneously named them as VPA and 2-ene-VPA glycinamides, rather than valproyl and 2-ene-valproyl glycine; the latter names are in accord with the method of preparation and the mass spectral data reported by Granneman, et al.

SUMMARY OF INVENTION

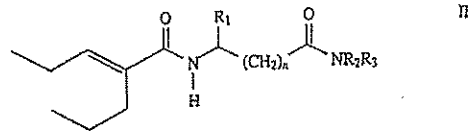
One object of the present invention is to provide a compound having the structure:



I

wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3.

Another object of the invention is to provide a compound having the structure:



II

wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3.

BRIEF DESCRIPTION OF THE DRAWINGS

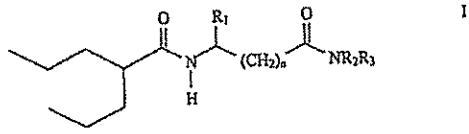
A more complete understanding of the invention and many of its advantages will become apparent by reference to the detailed description which follows when considered in conjunction with the accompanying figures wherein:

FIG. 1 illustrates performance in the passive avoidance test of rats treated with the indicated drugs for the duration of 28 days at the following daily oral doses: Compound 1, 200 mg/kg; VPA, 500 mg/kg. Tests were performed on day 10 after drug treatment. Latency, in seconds, represents response time to entry into dark compartment. Maximum latency is 300 sec. Longer latencies represent improved performance. Bars represent mean standard error (SEM).

FIG. 2 illustrates performance in the active avoidance test of rats treated with the indicated drugs for the duration of 28 days at the following daily oral doses: Compound 1, 200 mg/kg, VPA, 500 mg/kg. Test was performed on days 16–17 (session 1) and 22–23 (session 2) after initiation of drug treatment. Better performance is indicated by an increase in avoidance score, a decrease in latency time, and an increase in the number of crossings.

DESCRIPTION OF THE INVENTION

Compounds of particularly high activity and low toxicity result from the coupling of VPA at the carboxyl group with amino acid amides, and have the general structure I. The present invention provides a compound having the structure:



I

wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3.

In one embodiment, the invention provides the compound of formula I hereinabove shown wherein the C_1–C_6 alkyl group is a linear chain alkyl group. In another embodiment, the invention provides the compound of formula I hereinabove shown wherein the C_1–C_6 alkyl group is a branched chain alkyl group. In yet another embodiment, the invention provides the compound of formula I hereinabove shown wherein the aralkyl group is a benzyl, alkylbenzyl, hydroxybenzyl, alkoxycarbonylbenzyl, aryloxycarbonylbenzyl, carboxybenzyl, nitrobenzyl, cyanobenzyl, or halobenzyl group. In still another embodiment, the invention provides the compound of formula I wherein the aryl group is a phenyl, naphthyl, anthracenyl, pyridinyl, indolyl, furanyl, alkylphenyl, hydroxyphenyl, alkoxycarbonylphenyl, aryloxycarbon-

ylphenyl, nitrophenyl, cyanophenyl, halophenyl group, mercaptophenyl, or aminophenyl group.

In preferred embodiments, examples of the compound according to the invention include:

N-(2-n-propylpentanoyl)glycinamide;

N-(2-n-propylpentanoyl)glycine-N'-methylamide;

N-(2-n-propylpentanoyl)glycine-N'-butylamide;

N-(2-n-propylpentanoyl)leucinamide;

N-(2-n-propylpentanoyl)alanine-N'-benzylamide;

N-(2-n-propylpentanoyl)alaninamide;

N-(2-n-propylpentanoyl)-2-phenylglycinamide;

N-(2-n-propylpentanoyl)-4-aminobutyramide;

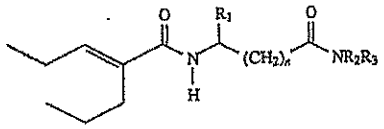
N-(2-n-propylpentanoyl)-β-alaninamide;

N-(2-n-propylpentanoyl)threoninamide; and

N-(2-n-propylpentanoyl)glycine-N',N'-dimethylamide.

In addition, novel compounds having the general structure II exhibiting high activity and low toxicity are related to those having general structure I, except for having a double bond in the 2-position.

The invention therefore provides a compound having the structure:



II

wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3.

In one embodiment, the invention provides the compound of formula II hereinabove shown wherein the C_1–C_6 alkyl group is a linear chain alkyl group. In another embodiment, the invention provides the compound of formula II hereinabove shown wherein the C_1–C_6 alkyl group is a branched chain alkyl group. In still another embodiment, the invention provides the compound of formula II hereinabove shown wherein the aralkyl group is a benzyl, alkylbenzyl, hydroxybenzyl, alkoxycarbonylbenzyl, aryloxycarbonylbenzyl, carboxybenzyl, nitrobenzyl, cyanobenzyl, or halobenzyl group. In yet another embodiment, the invention provides the compound of formula II hereinabove shown wherein the aryl group is a phenyl, naphthyl, anthracenyl, pyridinyl, indolyl, furanyl, alkylphenyl, hydroxyphenyl, alkoxycarbonylphenyl, aryloxycarbonylphenyl, nitrophenyl, cyanophenyl, halophenyl group, mercaptophenyl, or aminophenyl group.

In preferred embodiments, examples of the compound of formula I according to the invention include:

N-(2-n-propylpent-2-enoyl)glycinamide;

N-(2-n-propylpent-2-enoyl)alaninamide; and

N-(2-n-propylpent-2-enoyl)glycine-N'-methylamide.

The invention further provides a pharmaceutical composition which comprises any compound hereinabove shown in a therapeutically effective amount and a pharmaceutically acceptable carrier. The invention provides a pharmaceutical composition wherein the therapeutically effective amount is an amount from about 10 to about 500 mg. The invention encompasses a pharmaceutical composition as hereinabove described wherein the carrier is a solid and the composition is a tablet. The invention also encompasses a pharmaceutical composition as hereinabove described wherein the carrier is a gel and the composition is a suppository. The invention further encompasses a pharmaceutical composition as hereinabove described wherein the carrier is a liquid and the composition is a solution.

The invention provides a method of treating a subject afflicted with epilepsy which comprises administering to the subject an amount of the compound according to the invention effective to treat epilepsy in the subject.

The invention also provides a method of treating a subject afflicted with affective illness which comprises administering to the subject an amount of the compound according to the invention effective to treat the affective illness in the subject.

The invention additionally provides a method of treating a subject afflicted with cognitive disorders which comprises administering to the subject an amount of the compound according to the invention effective to treat cognitive disorders in the subject.

The invention further provides a method of treating a subject afflicted with neurodegenerative disease which comprises administering to the subject an amount of the compound according to the invention effective to treat neurodegenerative disease in the subject.

The invention also provides a method of treating a subject afflicted with dyskinesiae which comprises administering to the subject an amount of the compound according to the invention effective to treat dyskinesiae in the subject.

The invention still further provides a method of treating a subject afflicted with neurotoxic injury which comprises administering to the subject an amount of the compound according to the invention effective to treat neurotoxic injury in the subject.

The invention provides a method of alleviating convulsions in a subject afflicted with epilepsy which comprises administering to the subject an amount of the compound according to the invention effective to alleviate convulsions in the subject.

The invention also provides a method of treating a subject afflicted with stroke which comprises administering to the subject an amount of the compound according to the invention effective to treat stroke in the subject.

The invention additionally provides a method of treating a subject afflicted with brain ischemia which comprises administering to the subject an amount of the compound according to the invention effective to treat brain ischemia in the subject.

The invention still further provides a method of treating a subject afflicted with head trauma injury which comprises administering to the subject an amount of the compound according to the invention effective to treat head trauma injury in the subject.

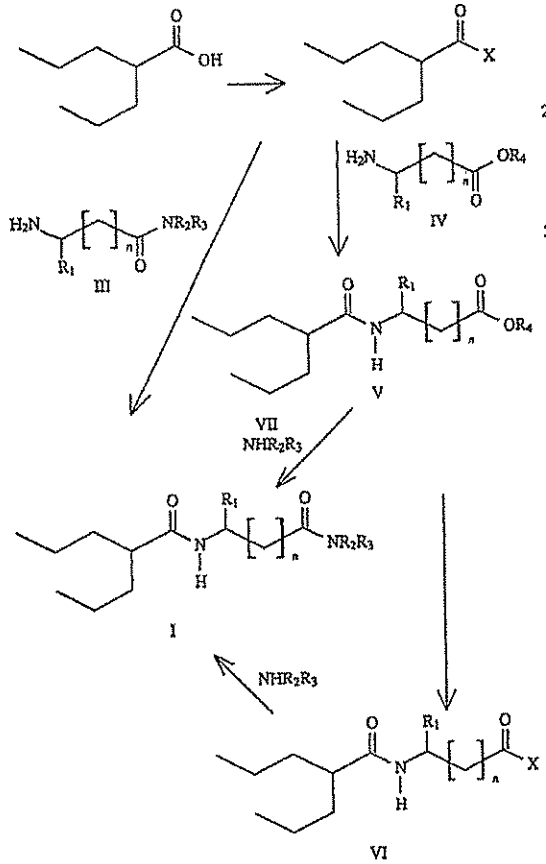
The compounds of general formulas I and II are potent anticonvulsant agents in conventional models of human epilepsy. Several of the compounds have a surprisingly better therapeutic profile than milacemide, VPA, VPA amide analogs or N-valproyl glycine. Furthermore, they may also be useful in the treatment of other CNS dysfunctions.

Suprisingly, the compounds of the invention are highly effective in the MES (maximal electroshock), electrical kindling model, and scMet (subcutaneous pentylenetetrazol) tests. The median effective doses (ED_{50}) of the agents claimed herein are considerably lower than those required to produce neurological impairment.

Therefore, results in animal models distinguish the compounds of the present invention from other antiepileptic agents and indicate that some of the disclosed compounds are effective against generalized and partial seizures, in addition to other forms of epilepsy, including absence seizures.

Some of the compounds of this invention possess chiral centers. It is a further embodiment of this invention that these compounds may comprise substantially pure D or L enantiomers or racemic mixtures. It is to be understood that compounds of the general formula II may be of the E-(trans) or Z-(cis) geometric configuration, or a mixture thereof.

The compounds of general formula I are diamides of valproic acid and may be prepared via conventional amidation processes, e.g., by reacting an activated form of the aforementioned acid either with an amino acid amide of the general formula III, where R_1, R_2, R_3 are the same or different and may be a hydrogen, an alkyl group (C_1–C_6), an aralkyl group or aryl group, and n=0 to 3, or with an amino acid derivative of the general formula IV, in which R_1 and n are the same as for III, and R_4 is hydrogen or a C_1–C_3 alkyl group. The resultant valproyl amino acid derivative V (wherein R_4 is a lower alkyl group) is reacted with amines of the general formula VII, or first activated (wherein R_4 is hydrogen), and the activated form of the acid, VI, is then reacted with VII.



R_4 = H or C_1–C_3 alkyl
X = halide or activated ester, e.g., N-oxysuccinimide

Thus, compounds I and V may be prepared in a biphasic system consisting of a basic aqueous solution of amino acid amides III or amino acid esters IV and a solution of valproyl chloride in an inert water-immiscible organic solvent, e.g. dichloromethane or toluene, at a temperature ranging between 0° and 50° C., preferably at 0°–10° C., for a period of 1 to 24 hrs, preferably 1 to 5 hrs.

The basic substance employed for the purpose may be either alkali, such as sodium hydroxide, potassium hydroxide, or potassium carbonate, or an aliphatic or aromatic tertiary amine, preferably triethylamine, and must be present in a quantity sufficient to neutralize the hydrohalic acid formed during the reaction.

Compounds I and V may also be prepared by reacting an activated ester of VPA with amino acid amides III or amino acid ester IV. Thus, VPA is reacted with an activating agent, e.g. , N-hydroxysuccinimide, pentafluorophenol, pentachlorophenol, or 1-hydroxybenzotriazole, in the presence of a dehydrating reagent such as a dialkylcarbodiimide, e.g., dicyclohexylcarbodiimide, diisopropylcarbodiimide, or N-(dimethylaminopropyl)-N'-ethylcarbodiimide, at a temperature ranging from 0°–50° C., preferably at 0°–25° C., in an inert solvent, such as tetrahydrofuran, dioxane, 1,2-dimethoxyethane, dichloromethane, or N,N-dimethylformamide. The resulting activated ester may be isolated and purified, or used directly in situ. The activated ester, whether purified or used directly, is reacted with III or IV, under the same conditions leading to condensation as detailed hereinabove.

The reaction of compounds V with amines R_2R_3NH may be carried out in a wide variety of organic solvents, including in an aprotic solvent which is a saturated or aromatic hydrocarbon, such as hexane, benzene, or petroleum ether, or a halogenated solvent, such as chloroform or dichloromethane, in a protic or alcoholic solvent, such as methanol or ethanol, or water. Preferably, the solvent is methanol. The reaction proceeds effectively at a temperature ranging from ambient to reflux, but preferably at 50°–70° C.

Compounds III may be used either as free bases or as their addition salts, formed by treatment of the free bases with an inorganic acid, such as tetrafluoroboric acid, hydrochloric acid, phosphoric acid, or sulfuric acid, or with an organic acid, such p-toluenesulfonic acid, acetic acid, or benzoic acid. Compounds III may be either a pure enantiomeric form, whether of D or L configuration, or a racemic mixture.

The amino acid amides and esters of general formulas III and IV are either commercially available or, alternatively, prepared from appropriate precursors, as detailed in the following examples.

The compounds of general formula II are diamides of valproenoic acid and may be prepared from the latter analogously to the compuonds of the general formula I.

Valproenic acid [(E)-2-ene valproic acid] may be prepared according to procedures known in the art. G. Taillandier, et al., *Arch. Pharm.* (Weinheim), 310, 394 (1977); C. V. Vorhees, et al., *Teratology*, 43, 583 (1991); R. C. Neuman, Jr., and G. D. Holmes, *J. Amer. Chem. Soc.*, 93, 4242 (1971).

In the practice of the invention, the amount of the compound incorporated in the pharmaceutical composition may vary widely. Factors considered when determining the precise amount are well known to those skilled in the art. Examples of such factors include, but are not limited to, the subject being treated, the specific pharmaceutical carrier, and route of administration being employed and the frequency with which the composition is to be administered. A pharmaceutical composition in unit dose form for treatment of the disorders listed hereinabove comprises 10 to 500 mg of the active ingredient.

In a preferred embodiment, the compound is administered in a pharmaceutical composition which comprises the compound and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutically accepted carriers, such as a phosphate-buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated

tablets, and capsules. An example of an acceptalbe triglyceride emulsion useful in the intravenous and intraperitoneal administration of the compounds is the triglyceride emulsion commercially known as Intralipid®.

Typically, such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients.

In the practice of the invention, the administration of the pharmaceutical composition may be effected by any of the well known methods including, but not limited to, oral, intravenous, intraperitoneal, intramuscular or subcutaneous or topical administration. Topical administration can be effected by any method commonly known to those skilled in the art and include, but are not limited to, incorporation of the pharmaceutical composition into creams, ointments, or transdermal patches.

The following Experimental Details are set forth to aid in an understanding of the invention, and are not intended, and should not be construed, to limit in any way the invention set forth in the claims which follow thereafter.

EXAMPLE 1

N-(2-n-Propylpentanoyl)glycinamide (compound 1).

A solution of valproyl chloride (108 g, 0.66 mole) in CH_2Cl_2 (500 ml) was added dropwise to an ice-cooled solution of glycinamide. HCl (72 g, 0.65 mole), and Et_3N (138 g, 1.37 mole) in water (200 ml). Cooling was discontinued and the two-phase mixture was stirred at RT for 3 hrs, cooled to 5°–8° C., and acidified to pH 2 by means of 1N HCl. The solid was collected by filtration, slurried in water (300 ml), filtered, dried and crystallized from EtOAc, affording 75 g (0.375 mole, 50%) of the title compound as a white crystalline solid, mp 127° C.

Anal. calc. for $C_{10}H_{20}N_2O_2$: C, 59.97; H, 10.06 N, 13.99; Found: C, 60.09; H, 10.25; N, 14.00.

^{1}H NMR δ ($CDCl_3$): 6.72 (br s, 1H, $CONH_2$), 6.65 (br t, 1H, CONH), 5.75 (br s, 1H, $CONH_2$), 3.98 (d, 2H, gly $C\alpha H_2$), 2.18 (m, 1H, Pr_2CH), 1.57, 1.40 (m, 4H, CH_3CH_2 $\underline{CH_2}$), 1.29 (m, 4H, $CH_3\underline{CH_2CH_2}$), 0.89 (t, 6H, CH_3) ppm.

MS: 201 (MH^+, 100), 184 (MH^+–NH_3, 24).

IR: 3240, 3312, 3181, 2953, 2932, 2872, 1676, 1630, 1549, 431, 1325, 1271, 1221 cm^{-1}

EXAMPLE 2

N-(2-n-Propylpentanoyl)leucinamide.

The title compound was prepared from valproyl chloride (2.0 g, 12.3 mmole) and DL-leucinamide hydrochloride (2.0 g, 12.05 mmole), according to the procedure described in Ex. 1. 2.36 g (9.2 mmole, 76%) of a white crystalline solid, mp 151°–2° C., was thus obtained.

Anal. calc. for $C_{14}H_{28}N_2O_2$: C, 65.58; H, 11.01; N, 10.93; Found: C, 65.28; H, 10.89; N, 10.86.

^{1}H NMR δ (DMSO): 7.85 (br d, 1H, CONH), 7.20 (br s, 1H, $CONH_2$), 6.89 (br s, 1H, $CONH_2$), 4.27 (m, 1H, leu CαH), 2.25 (m, 1H, Pr_2CH), 1.60, 1.42, 1.20 (m, 11H, CH_3 $\underline{CH_2CH_2}$, $Me_2\underline{CHCH_2}$), 0.88 (d, 3H, leu Me), 0.83 (d, 3H, leu Me), 0.83 (br t, 6H, Me) ppm.

MS: 257 (MH+, 100), 240 (MH^+–NH_3, 32).

IR: 3410, 3300, 2955, 2925, 1720, 1655, 1645, 1540, 1260 cm^{-1}.

EXAMPLE 3

N-(2-n-propylpentanoyl)-2-phenylglycinamide.

A solution of valproyl chloride (1.95 g, 12 mmole) in 1,2-dimethoxyethane (DME, 30 ml) was added to an ice-cooled suspension of phenylglycinamide (1.80 g, 12 mmole, prepared from DL-phenylglycinonitrile, Ger. off. 2637204) and Et3N (2.4 g, 24 mmole) in DME (35 ml). The reaction mixture was stirred under a nitrogen atmosphere for 24 hrs at RT, and the resultant product was collected by filtration, washed with cold hexane (50 ml) and taken into EtOAc/H20 (200 ml:175 ml). The organic layer was separated, washed successively with satd. NaHC03, 0.1N HCl and satd. NaCl, dried and evaporated to dryness. The crude product was crystallized from EtOAc, affording 2.50 g (9.06 mmole, 75%) of the title compound as a white crystalline solid, mp 190°–1° C.

Anal. calc. for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14; Found: C, 68.26; H, 8.57; N, 9.96.

^{1}H NMR δ (DMSO): 8.36 (br d, 1H, CONH), 7.65 (br s, 1H, CONH), 7.46–7.22 (m, 5H, Ph), 7.10 (br s, 1H, $CONH_2$), 5.46 (d, 1H, Ph—$\underline{CH}$), 2.44 (m, 1H, $Pr_2\underline{CH}$), 1.40, 1.22, 1.10 (m, 8H, $CH_3\underline{CH_2CH_2}$), 0.85 (t, 3H, Me), 0.78 (t, 3H, Me) ppm.

MS: 277 (MH^+, 56), 201 (100).

IR: 3400, 3300, 2950, 2910, 1735, 1685, 1560, 1400 cm^{-1}.

EXAMPLE 4

N-(2-n-Propylpentanoyl)alanine methyl ester.

A solution of DL-alanine methyl ester hydrochloride (13.7 g, 98 mmole) and Et_3N (20.2 g, 200 mmole) in water (50 ml) was added dropwise to an ice-cooled solution of valproyl chloride (15.0 g, 92 mmole) in CH_2Cl_2 (150 ml). After completion of addition the reaction mixture was stirred for 4 hrs. at RT. The layers were then separated and the aqueous layer extracted with CH_2Cl_2. The combined organic phases were washed successively with water, satd. $NaHCO_3$, 0.1N HCl and satd. NaCl, dried and evaporated to dryness. The residue was treated with hexane (60 ml), and the resultant solid was collected by filtration, washed with hexane and dried to give 14.2 g (62 mmole, 63%) of the title compound as a white solid, mp 72°–3° C.

^{1}H NMR δ ($CDCl_3$): 6.02 (br d, 1H, NH), 4.63 (quintet, 1H, ala CαH), 3.75 (s, 3H, OMe), 2.08 (m, 1H, $Pr_2\underline{CH}$), 1.6, 1.4, 1.32 (m, 8H, $CH_3\underline{CH_2CH_2}$), 1.40 (d, 3H, ala Me), 0.89 (t, 6H, Me) ppm.

MS: 230 (MH^+, 100), 127 (7), 104 (16).

IR: 3300, 2925, 1740, 1630, 1540 cm^{-1}.

EXAMPLE 5

N-(2-n-Propylpentanoyl)glycine methyl ester.

The title compound was prepared from valproyl chloride (19.34 g, 119 mmole) and glycine methyl ester hydrochloride (15.0 g, 119 mmole), according to the procedure described in Ex. 4.22 g (102 mmole, 86%) of an off-white solid, mp 68° C., was thus obtained.

^{1}H NMR δ ($CDCl_3$): 5.97 (br t, 1H, NH), 4.06 (d, 2H, gly CH_2), 3.76 (s, 3H, OMe), 2.14 (m, 1H, $Pr_2\underline{CH}$), 1.60, 1.45–1.25 (m, 8H, $CH_3\underline{CH_2CH_2}$), 0.90 (t, 6H, Me) ppm.

MS: 216 (MH^+, 100), 127 (13).

IR: 3300, 2945, 2920, 1765, 1650, 1550, 1220 cm^{-1}.

EXAMPLE 6

N-(2-n-Propylpentanoyl)alaninamide.

Aqueous ammonia (25%, 50 ml) was added dropwise to a solution of N-(2-propylpentanoyl)alanine methyl ester (6.87 g, 30 mmole) in methanol (20 ml), and the reaction

mixture was stirred under reflux for 4 hrs. The solid which precipitated upon cooling was filtered, washed with cold hexane, dried and crystallized from EtOAc to give 1.90 g (8.92 mmole, 30%) of the title compound as a white crystalline solid, mp 165°–166° C.

Anal calc. for $C_{11}H_{22}N_2O_2$: C, 61.64; H, 10.35; N, 13.08; Found: C, 61.35; H, 10.26; N, 13.32.

^{1}H NMR δ (DMSO): 7.84 (br d, 1H, CONH), 7.21 (br s, 1H, $CONH_2$), 6.92 (br s, 1H, $CONH_2$), 4.25 (quintet, 1H, ala CαH), 2.24 (m, 1H, Pr_2—CH), 1.42, 1.20 (m, 8H, $CH_3CH_2CH_2$), 1.17 (d, 3H, ala Me), 0.833 (t, 3H, Me), 0.827 (t, 3H, Me) ppm.

MS: 214 (M^+,1), 170 (M^+–$CONH_2$, 100).

IR: 3390, 3295, 1675, 1620 cm^{-1}.

EXAMPLE 7

N-(2-n-Propylpentanoyl)alanine-N'-benzylamide.

The title compound was prepared from N-(2-propylpentanoyl) alanine methyl ester (3.67 g, 16 mmole) according to the procedure described in Ex. 6, except that a methanolic solution of benzylamine (1.5 molar excess) was used, and the reaction mixture was stirred under reflux for 24 hours. 1.4 g (4.6 mmole, 29%) of the title compound as a white solid, mp 139° C., was thus obtained.

Anal calc. for $C_{18}H_{28}N_2O_2$: C, 71.01; H, 9.27; N, 9.21; Found: C, 70.88; H, 9.15; N, 9.24.

^{1}H NMR δ (DMSO): 7.25 (m, 6H, $PhCH_2NH$), 6.40 (br d, 1H, CONH), 4 61 (quintet, 1H, ala CαH), 4.39 (m, 2H, Ph—CH_2), 2.06 (m, 1H, Pr_2CH) 1.50, 1.25 (m, 8H, $CH_3CH_2CH_2$), 1.34 (d, 3H, ala Me), 0.87 (t, 3H, Me), 0.82 (t, 3H, Me) ppm.

MS: 304 (M^+,34), 198 (M^+–$PhCH_2NH$, 11), 171 (44).

IR: 3280, 2945, 2925, 1640, 1550, 1445 cm^{-1}.

EXAMPLE 8

N-(2-Propylpentanoyl)glycine-N'-methylamide.

The title compound was prepared from N-(2-propylpentanoyl)glycine methyl ester (5.0 g, 23.2 mmole) and 35% aqueous methylamine (56.4 mmole), according to the procedure described in Ex. 7. 2.86 g (13.4 mmole, 58%) of a white crystalline solid, mp 146° C., was thus obtained.

Anal. calc. for $C_{11}H_{22}N_2O_2$: C, 61.65; H, 10.35; N, 13.07; Found: C, 61.36; H, 10.14; N, 12.78.

^{1}H NMR δ (DMSO): 7.99 (br t, 1H, $CONHCH_2$), 7.69 (m, 1H, $CONHCH_3$), 3.62 (d, 2H, gly CH_2), 2.58 (d, 3H, NH Me), 2.22 (m, 1H, Pr_2CH), 1.45, 1.22 (m, 8H, $CH_3CH_2CH_2$), 0.83 (t, 6H, Me) ppm.

MS: 215 (MH^+,100), 197 (MH^+–H_2O, 23), 184 (MH^+–$MeNH_2$, 65), 127 (8).

IR: 3300, 2960, 2920, 2870, 1660, 1630, 1555, 1440, 1420 cm^{-1}.

EXAMPLE 9

N-(2-n-Propylpentanoyl)glycine-N'-butylamide.

The title compound was prepared from N-(2-propylpentanoyl)glycine methyl ester (5.0 g, 23.0 mmole) and butylamine (4.1 g, 55.0 mole), according to the procedure described in Ex. 7. 2.2 g (8.5 mmole, 37%), mp 101° C., was thus obtained.

Anal. calc. for $C_{14}H_{28}N_2O_2$: C, 65.58; H, 11.01; N, 10.93; Found: C, 65.87; H, 11.23; N, 11.38.

^{1}H NMR δ (DMSO): 7.99 (br t, 1H, NH), 7.65 (br t, 1H, NH), 3.63 (d, 2H, gly CH_2), 3.05 (m, 2H, $CH_3CH_2CH_2CH_2NH$), 2.22 (m, 1H, Pr_2CH), 1.50–1.16 (m, 12H, CH_2CH_2, $CH_3CH_2CH_2CH_2NH$), 0.85 (t, 3H, $CH_3CH_2CH_2NH$), 0.83 (t, 3H, $CH_3CH_2CH_2$) ppm.

MS: 257 (MH^+, 100), 184 (MH^+–$C_4H_9NH_2$, 19).

IR: 3300, 2940, 1660, 1635, 1555, 1470, 1435, 1300 cm^{-1}.

EXAMPLE 10

N-2-n-Propylpentanoyl)glycine-N'-methylamide.

The title compound was prepared from valproyl chloride (404 mg, 2.5 mmole) and 2-amino-N-methylacetamide (220 mg, 2.5 mmole, prepared from glycine methyl ester hydrochloride and methylamine), according to the procedure described in Ex. 1. 318 mg (1.49 mmole, 59%) of a white crystalline solid was thus obtained, identical to the product described in Ex. 8.

EXAMPLE 11

N-(2-n-Propylpentanoyl)-4-aminobutyramide.

To an ice-cooled solution of N-(2-propylpentanoyl)-4-aminobutyroyl chloride (prepared from N-(2-propylpentanoyl)-4-aminobutyric acid and $SOC_{12, s.g}$ g, 24.0 mmole) in dioxane (25 ml), was added dropwise conc. NH_4OH (34 ml) over 1 hr. The reaction mixture was then stirred at RT for 20 hrs and evaporated to dryness under reduced pressure. The residue was taken up in an H_2O (20 ml) and EtOAC (30 ml) mixture, the mixture stirred vigorously for 5 min. The organic phase was separated, evaporated to dryness under reduced pressure, and the residue crystallized from EtOAc to give 1.4 g (6.1 mmole, 26%) of a crystalline solid, mp 138° C.

Anal calc for $C_{12}H_{24}N_2O_2$: C, 63.13; H, 10.60; N, 12.27; Found: C, 63.12; H, 10.69; N, 12.54.

^{1}H NMR δ (DMSO): 7.81 (br t, 1H, NH), 7.26 (br s, 1H, $(CH_2)3CONH_2$), 6.73 (br s, 1H, $(CH_2)_3CONH_2$), 3.02 (m, 2H, $CH_2CH_2CH_2CONH_2$), 2.11 (m, 1H, Pr_2CH), 2.03 (t, 2H, CH_2CONH_2), 1.58 (m, 2H, $CH_2CH_2CONH_2$), 1.42 (m, 2H, CH_2CHCO), 1.19 (m, 6H, CH_2CH_2CHCO), 0.84 (t, 6H, Me) ppm.

MS: 229 (MH_+, 100), 127 (17).

IR: 3405, 3300, 3190, 2960, 2935, 2880, 1660, 1655, 1635, 1550, 1445 cm^{-1}.

EXAMPLE 12

N-[2-n-Propylpent-(E)-2-enoyl]glycinamide.

A cold solution of glycinamide hydrochloride (6.63 g, 60 mmole) in water (18 ml) and Et_3N (12.7 g, 126 mmole) were added slowly to a stirred and ice-cooled solution of (E)-2-ene-valproyl chloride in toluene (40 ml). After completion of addition, the biphasic reaction mixture was stirred at ambient temperature for 3 hrs. Work-up and crystallization according to the procedure in Ex. 1 afforded 6.92 g (34.8 mmole, 58%) of the title compound as a white crystalline solid, mp 112° C.

Anal. calcd. for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.13; N, 14.13; Found: C, 60.53; H, 8.86; N, 14.04.

^{1}H NMR δ ($CDCl_3$): 6.97 (br s, 1H, $CONH_2$), 6.91 (br t, 1H, NH), 6.29 (t, 1H, vinyl), 6.05 (br s, 1H, $CONH_2$), 2.28 (m, 2H, CH_3CH_2CH=), 2.17 (m, 2H, $CH_3CH_2CH_2$), 1.42 (m, 2H, $CH_3CH_2CH_2$), 1.05 (t, 3H, Me), 0.93 (t, 3H, Me) ppm.

MS: 199 (MH^+, 83), 182 (MH^+–NH_3, 79), 125 (100).

IR: 3341, 3179, 2955, 2872, 1680, 1601, 1535, 1433, 1319 cm^{-1}.

EXAMPLE 13

N-[2-n-Propylpent-(E)-2-enoyl]alanine methyl ester.

The title compound was prepared from (E)-2-enevalproyl chloride (10.95 g, 68.1 mmole) and alanine methyl ester hydrochloride (10.14 g, 72.6 mmole) according to the procedure described in Ex. 4. The crude product was crystallized from hexane to give 13.25 g (58.4 mmole, 86%) of a white crystalline solid, mp 25° C.

^{1}H NMR δ ($CDCl_3$): 6.30 (br d, 1H, NH), 6.23 (t, 1H, vinyl) 4.65 (m, 1H, ala CH), 3.76 (s, 3H, OMe), 2.29 (m, 2H, $CH_3\underline{CH_2}CH$═), 2.17 (m, 2H), 1.43 (d, 3H, ala CH_3), 1.43 (m, 2H, $CH_3\underline{CH_2}CH_2$), 1.04 (t, 3H, Me), 0.92 (t, 3H, Me) ppm.

MS: 228 (MH^+, 100), 196 (NH^++–NH_3, 100), 168 (30), 125 (76).

EXAMPLE 14

N-[2-n-Propylpent-(E)-2-enoyl]glycine-N'-methylamide.

The title compound was prepared from N-[2-n-propylpent-(E)-2-enoyl]glycine methyl ester (13.5 g, 63.9 mmole), prepared from 2-ene-valproyl chloride and glycine methyl ester hydrochloride as described in Ex. 5, and 35% aqueous methylamine (15 ml, 169.2 mmole), according to the procedure described in Ex. 7. The amide product was purified by column chromatography and crystallized from EtOAc to give 7.8 g (36.8 mmole, 58%) of a white crystalline solid, mp 68°–9° C.

Anal. caled. for $C_{11}H_2ON_2O_2$: C, 62.23; H, 9.50; N, 13.20; Found: C, 62.42; H, 9.50; N, 13.05.

^{1}H NMR δ (DMSO): 7.94 (br t, 1H, NH) 7.67 (m, 1H, $\underline{NH}CH_3$), 6.23 (t, 1H, vinyl), 3.65 (d, 2H, gly), 2.58 (d, 3H, $NH\underline{CH_3}$), 2.21 (m, 2H, $CH_3\underline{CH_2}CH$═), 2.13 (m, 2H, $CH_3CH_2\underline{CH_2}$), 1.32 (m, 2H, $CH_3\underline{CH_2}CH_2$), 0.99 (t, 3H, Me), 0.85 (t, 3H, Me) ppm.

MS: 213 (MH^+, 73), 195 (37), 182 (MH^+–CH^+3NH_2, 100), 125 (74).

IR: 3300, 2955, 2925, 1660, 1620, 1560, 1540, 1460 cm^{-1}.

EXAMPLE 15

N-[2-n-propylpent-(E)-2-enoyl]alaninamide.

The title compound was prepared from N-[2-n-propylpent-(E)-2-enoyl]alanine methyl ester (9.08 g, 40 mmole) and aqueous ammonia (67 ml), in a manner analogous to that described in Ex. 6, giving 5.0 g (59%) of a white crystalline solid, mp 141°–2° C.

Anal. calcd. for $C_{11}H_{20}N_2O_2$: C, 62.23; H, 9.50; N, 13.20; Found: C, 62.48; H, 9.25; N, 13.18.

^{1}H NMR δ (DMSO): 7.63 (d, 1H, NH) 7.25 (br s, 1H, $CONH_2$), 6.96 (br s, 1H, $CONH_2$), 6.18 (t, 1H, vinyl) 4.25 (m, 1H, ala CH), 2.21 (m, 2H, $CH_3\underline{CH_2}CH_2$), 1.31 (m, 2H, $CH_3\underline{CH_2}CH$═), 2.11 (m, 2H, $CH_3CH_2\underline{CH_2}$), 1.31 (m, 2H, $CH_3\underline{CH_2}CH_2$), 1.23 (d, 3H, ala CH_3), 0.99 (s, 3H, Me), 0.84 (s, 3H, Me) ppm.

MS: 213 (MH^+, 74), 196 (MH^+–NH_3, 100), 125 (76).

IR: 3725, 3180, 2950, 1700, 1650, 1605, 1530, cm^{-1}

EXAMPLE 16

N-(2-n-Propylpentanoyl)-β-alaninamide.

A mixure of N-(2-n-propylpentanoyl)-β-alanine ethyl ester (4.45 g, 18.29 mmole), prepared from valproyl chloride and β-alanine ethyl ester hydrochloride according to the procedure described in Ex. 4, dry formamide (2.74 g, 61.27 mmole) and anhydrous THF (9.2 ml) was heated to 100° C., and a freshly prepared solution of sodium methoxide (12.7 mmole) in MeOH (2.93 ml) was added dropwise over 20 min. The mixture was heated at 100° C. for 4 hours and isopropanol (100 ml) was added. The suspension was heated to reflux, filtered, and the filtrate was evaporated to dryness. The residue was dissolved in a refluxing mixture of water and EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (4×100 ml). The combined organic layers were washed with water, dried, and evaporated to dryness. The crude product (2.5 g) was crystallized from EtOAc to give 2.20 g (10.28 mmole, 56%) of a white solid, mp 167°–8° C.

Anal. calcd. for $C_{11}H_{22}N_2O_2$: C, 61.64; H, 10.35; N, 13.08. Found: C, 61.41; H, 10.16; N, 12.91.

^{1}H NMR δ (DMSO): 7.82 (br t, 1H, CONH), 7.29 (br s, 1H, $CONH_2$), 6.79 (br s, 1H, $CONH_2$), 3.20 (q, 2H, β-ala), 2.21 (t, 2H, α-ala), 2.12 (m, 1H, $(Pr)_2\underline{CH}$), 1.41, 1.18 (m, 8H, $CH_3\underline{CH_2}\underline{CH_2}$), 0.83 (t, 6H, Me) ppm.

MS: 215 (MH^+, 100), 197 (MH^+–$NH_{3,\ 69}$), 172 (13), 127 (3).

IR: 3389, 3303, 3202, 2957, 2928, 1653, 1634, 1551, 1456, 1439 cm^{-1}.

EXAMPLE 17

N-(2-n-Propylpentanoyl)threoninamide.

A solution of valproyl chloride (3.15 g, 19.4 mmole) in anhydrous 1,2-dimethoxyethane (DME, 48 ml) was added slowly to a suspension of threoninamide hydrochloride (3.0 g, 19.4 mmole) and Et_3N (3.88 g, 38.8 mmole) in anhydrous DME (60 ml) at 10°–15° C. The reaction mixture was stirred for 24 hours at RT under N_2; the solvent was removed under reduced pressure, and the residue was worked up in a manner analogous to that in Ex. 16. The product was crystallized from EtOAc to give 1.0 g (4.1 mmole, 21%) of a white solid, mp 172°–4° C.

Anal. calcd. for $C_{12}H_{24}N_2O_3$: C, 58.99, H, 9.90; N, 11.47; Found: C, 58.12; H, 9.42; N, 11.43.

^{1}H HMR δ (DMSO): 7.58 (d, 1H, CONH), 7.05 (br s, 2H, $CONH_2$), 4.84 (d, 1H, OH), 4.18 (dd, 1H, α-thr), 3.99 (m, 1H, β-thr), 2.35 (m, 1H, $Pr_2\underline{CH}$), 1.44, 1.22 (m, 8H, CH_3 $\underline{CH_2}\underline{CH_2}$), 1.02 (d, 3H, Me-thr), 0.85 (t, 3H, Me), 834 (t, 3H, Me) ppm.

MS: 245 (MH^+, 37), 228 (MH^+–NH_3, 100).

IR: 3405, 3281, 2957, 2930, 2854, 1688, 1665, 1624, 1549 cm^{-1}.

EXAMPLE 18

N-(2-n-Propylpentanoyl)glycine-N',N'-dimethylamide.

N-(2-n-Propylpentanoyl)glycine methyl ester (6.0 g, 29.9 mmole) prepared from valproyl chloride and glycine methyl ester hydrochloride according to the procedure in Ex. 4 was dissolved in MeOH (15 ml) and 40% aqueous dimethylamine (11 ml) was added dropwise. The reaction mixture was refluxed for 19 hr and evaporated to dryness. The reaction mixture was treated with hot ethyl acetate, cooled, and filtered. The filtrate was washed consecutively with sat. $NaHCO_3$ and sat. NaCl solution, dried and evaporated to dryness. The solid residue was crystallized from ethyl acetate/hexane to give 1.50 g of a white solid, mp 78°–80° C.

Anal. calcd. for $C_{12}H_{24}N_2O_2$: C, 63.12, H, 10.59; N, 12.27. Found: C, 62.80, H, 10.64; N, 11.93.

^{1}HNMR δ (DMSO): 7.73 (br t, 1H, CONH), 3.79 (d, 2H, gly), 2.84 (s, 3H, Me), 2.72 (s, 3H, Me), 2.16 (m, 1H, $(Pr)_2$ $\underline{CH}$), 1.34 (m, 2H), 1.12 (m, 6H), 0.74 (t, 6H, Me) ppm.

MS: 229 (MH^+, 100), 184 (18).

IR: 3314, 2951, 2924, 2872, 1662, 1630, 1522, 1466 cm^{-1}.

EXAMPLE 19

Biological Activity of N-(2-Propylpentanoyl)glycinamide.

All compounds provided herein were screened for their ability to protect against chemically and electrically induced convulsions, in at least two different models of epilepsy. The first model, the subcutaneous pentylenetetrazol (s.c. Met) seizure threshold test, is a standard screening procedure to show efficacy for agents against absence seizures. The second model, the maximal electroshock (MES) test, is used to show efficacy for antiepileptic agents against generalized seizures. In these studies, convulsions were inhibited or prevented in mice after intraperitoneal (i.p.) administration and/or in rats after oral (p.o.) administration of the compounds.

N-(2-Propylpentanoyl)glycinamide (hereinafter compound 1) was further tested in two additional models. The third model, electrical kindling of rats, has been known to show efficacy of antiepileptic agents against complex partial seizures that evolve into generalized motor seizures. In these tests, rats were electrically stimulated via corneal electrodes twice daily for approximately 5 days and then once daily for an additional 10 days. Once the seizure criteria, as described by R. J. Racine, et al., *Electroenceph. Clin. Neurophysiol.*, 32: 281–294 (1972), were met, the test substance was administered p.o. to rats, and the rat electrically stimulated, and observed for the presence or absence of a seizure. In addition, compound 1 was also tested in the subcutaneous bicuculline model (s.c. Bic). For detailed procedures of all the above test models, see E. A Swinyard, et al , in "Antiepileptic Drugs," ed. by R. H. Levy, et al., Raven Press, New York, at 85–100 (1989) and Racine, Id.

Compound 1 showed anticonvulsant activity in rodents in all of the above mentioned tests (MES, s.c. Met, s.c. Bic, and electrical kindling models). The ED50 (rat, p.o.) in the MES model was 73 mg/kg (Table 1). This value is seven times lower (more efficacious) than that found for VPA, and approximately twice that found for phenytoin (Table 1; see E.A. Swinyard, et al., id.). Further, in the electrically kindled rat model, compound 1 (administered p.o.) prevented seizures with an ED_{50} of 162 mg/kg (Table 1). The results are therefore indicative of compound 1 having an efficacy against generalized seizures and complex partial seizures which evolve into generalized motor seizures.

In addition, in the s.c. Bic model, compound 1 provided full protection from seizures in mice, at a dose that was approximately that of literature values for the ED_{50} for VPA. Literature values also show that phenytoin, considered the drug of choice for partial and generalized tonic-clonic seizures, is not effective in this model. See B. J. Wilder and R. J. Rangel, in "Antiepileptic Drugs," ed. by R. H. Levy, et al., Raven Press, New York, at 233–239 (1989).

In the s.c. Met model (mice, i.p.), the ED_{50} for compound 1 was 127 mg/kg (Table 1) as compared to the literature value of 146 mg/kg for VPA. These results further indicate efficacy for compound 1 against absence seizures as well.

EXAMPLE 20

Neurotoxicity of Compound 1.

Neurotoxicity of the claimed agents was also assessed in mice (i.p. adminstration) by the rotorod ataxia test and also in some cases in rats (p.o. administration) by the positional sense test and gait and stance test. See E. A. Swinyard, et al., in "Antiepileptic Drugs," ed. by R. H. Levy, et al., Raven Press, New York, at 85–100 (1989). None of the agents provided in the invention showed neurotoxicity in mice at the test dose of 100 mg/kg. Compound 1 had a median neurological toxic dose (TD_{50}) in rats of more than 1000 mg/kg. By comparison, the TD_{50} for VPA was 280 mg/kg. In mice, the difference between TD_{50} values between compound 1 and VPA was smaller, but still significantly higher for compound 1 (less neurotoxic) (Table 1). The protective index (PI, $PI=TD_{50}/ED_{50}$) for compound 1 in rats tested in the MES test is more than 23 times greater than that found for VPA (Table 1). These results are shown to indicate that there is a larger therapeutic dose range that can be administered before neurological side effects are usually observed.

The median lethal dose (LD_{50}) of compound 1 in mice (i.p. administration) is more than 4,000 mg/kg. This value is in contrast to VPA whose LD_{50} in the same test was 658 mg/kg. The results, therefore, indicate that compound 1 is considerably less toxic than VPA.

EXAMPLE 21

Neurological Activity of Compound 1.

A major neurological side effect observed in patients on treatment with antiepileptic agents is cognitive impairment. Present data further indicate that at the minimum dose required to provide full protection from seizures induced in rats in the MES test, compound 1 results in less cognitive impairment than VPA. Results from the models used are taken as indicators of major constituents of human cognition.

The studies test for the level of motivation, association and short and long-term memory. The specific studies were the effect of compound 1 on the performance of rats in the locomotor test and passive and active response tests. In the cognitive studies below, doses used for compound 1 and VPA were the minimum doses which give full protection against seizures in the MES test (Compound 1=200 mg/kg and VPA=500 mg/kg).

In the locomotor test, motor activity was recorded 8 to 9 days after the beginning of drug treatment. Locomotion scores were recorded in cages (25×26cm) having a grid of infra-red beams at 4 cm intervals. Two categories of movements were recorded: small movements (those originating in stationary activities such as grooming and scratching), and big movements (those resulting in ambulation and recorded as the simultaneous crossing of more than two beams). Since rats are nocturnal animals, recordings were usually made between 18:00 PM–6:00 AM.

The results in the locomotor test (Table 2) show no significant difference in motor activity between the control and compound 1.

To measure passive avoidance responses, tests were performed on days 10, 12, 14, 20, and 26 after initiation of drug treatment. The apparatus consisted of a lit chamber that can be separated from a dark chamber by a sliding door. In the experiment, a rat is placed in a lit chamber for 30 sec, the door is then opened and the rat moves into the dark chamber with latency that is recorded. Upon entry into the dark chamber, the door is shut and a 0.3 mA footshock is delivered for 3 sec. Retention of the experience is determined after 48 hours by repeating the test and recording the latency. The maximum latency was arbitrarily assigned the value of 300 sec. Longer latencies are taken as a measure of improved memory.

Results from this study show that on day 16 of the test, the group receiving compound 1 retained their acquired knowledge to avoid the electric shock as well as the control group

(FIG. 1). The VPA-treated rats, however, were apparently affected by treatment, and performed much worse. These results suggest that VPA adversely affected memory, whereas compound 1 did not have this adverse effect.

The conditioned avoidance response (active avoidance test) of rats was determined in a Hugo-Basile automatic conditioning apparatus, which consists of a shuttle box with two separate floor grids. In this apparatus the rats are conditioned to jump from one side of the box to the other side. The conditioning is a 10 sec stimulus consisting of a light and electric buzzer. At the end of this stimulus the rats which do not jump to the other side of the box receive a 20 sec electroshock (50 V, 0.3 mA) from the grid floor. The rats that do jump to the other side of the box do not receive the shock. The session is then repeated with the same rats 7 days later. Experiments were carried out on days 16–17 and 22–23 from the start of drug treatment, and each rat received 60 trials with a 30 sec interval between each trial.

The following parameters were recorded: a) the number of potential shocks successfully avoided; b) the latency response in seconds for avoiding a potential shock; and c) the total number of crossings made throughout the trials. In this test, a better performance is indicated by an increase in the avoidance of an electric shock, a decrease in the latency time to jump to the other side of the cage, and an increase in the number of times the rats crossed to the other side of the cage.

Rats treated with compound 1 showed a significantly better performance than the VPA treated group. The performance of the animals treated with compound 1 was similar to that of the control group, whereas the VPA-treated rats had a worse performance (FIG. 2 and Table 3).

The tests stated hereinabove are consistent with the conclusion that compound 1 causes less cognitive impairment than VPA.

Based on the lower ED_{50} and on the higher TD_{50} and LD_{50} values of compound 1, as compared to those of VPA, the former may be considered to act by a unique mechanism, and not as a prodrug of VPA. Moreover, these results are quite unexpected in view of the fact that neither valproylglycine nor milacemide was active when tested in mice (i.p. administration at doses up to 300 mg/kg), in the MES and s.c. Met models.

TABLE 1

Anticonvulsant profile of the claimed and reference antiepileptic agents.

COMPOUND	COMPOUND 1 (mg/kg)	Phenytoin (mg/kg)	Valproic acid (mg/kg)	Carbamzaepine (mg/kg)
Rat p.o TD50 MES model	>1000	>3000	281	813
ED50	73	29.8	490	8.5
PI	>13.7	100	0.6	95.7
s.c. MET model				
ED50	—	N.E.	180	N.E.
PI	—	—	1.6	—
Electrical kindling model ED50	162	—	117	28.9
Mice i.p. TD50 MES model	369	65.5	426	71.6
ED50	152	9.5	272	
PI	2.4	6.9	1.6	8.1
s.c. MET model				
ED50	127	N.E.	149	N.E.
PI	2.9	—	2.9	

The anticonvulsant profile of compound I compared to literature values (for anticonvulsant activity whose experimental protocols were identical to those carried out in the current study) for the prototype anticonvulsant agents VPA and phenytoin. Convulsions were induced in mice and rats by subcutaneous administration of pentylenetetrazol (s.c. Met test) or by electrical stimulation (MES test). N.E. = not effective.

TABLE 2

Activity scores of rats chronically treated with compound 1.

	Day activity 14.00–20.00 h		Night activity 20.00–08.00 h	
Treatment	Big mov.	Total mov.	Big mov.	Total mov.
Control (7)	1939 ± 349	6391 ± 983	6124 ± 489	23750 ± 2075
compound 1 (7)	2402 ± 307	7749 ± 1188	7217 ± 765	22568 ± 2209

TABLE 2-continued

Activity scores of rats chronically treated with compound 1.

Treatment	Day activity 14.00–20.00 h		Night activity 20.00–08.00 h	
	Big mov.	Total mov.	Big mov.	Total mov.
Na Valproate 500 mg/kg (6)	2784 ± 352	8963 ± 1554	5832 ± 854	18876 ± 2039

Activity scores of drug-treated rats, measured in activity cages on days 8–9 after initiation of daily oral dosing with the given drug. Figures are number of crossings ± SEM. Number of rats per group are given in parenthesis.

TABLE 3

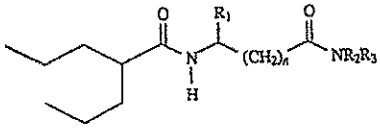
Active avoidance response of claimed and reference compounds.

	Drug treatment					
	Session I			Session II		
	Avoidance	Latency	Crossings	Avoidance	Latency	Crossings
Control (7)	9 ± 5	23 ± 3	32 ± 10	9 ± 5	25 ± 2	30 ± 10
compound 1 200 mg/kg (7)	14 ± 7	21 ± 3	38 ± 13	12 ± 7	22 ± 3	35 ± 9
Carbamizepine 15 mg/kg (4)	7 ± 4	27 ± 2	18 ± 10	2 ± 2	29 ± 1	11 ± 8
Na Valproate 500 mg/kg (6)	2 ± 3	28 ± 0.4	13 ± 2	6 ± 5	27 ± 2	16 ± 9

Scores in the active avoidance test (conditioned avoidance response) of rats treated with compound 1 and related drugs. The tests in the first session were performed on days 16–17 from initiation of drug administration. Those in session II were performed on days 22–23, that is 7 days following session I. Number of rats in a group are given in parenthesis.

What is claimed is:

1. A compound having the structure:



wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is equal to 0.

2. The compound of claim 1, wherein the C_1–C_6 alkyl group is a linear chain alkyl group.

3. The compound of claim 1, wherein the C_1–C_6 alkyl group is a branched chain alkyl group.

4. The compound of claim 1, wherein the aralkyl group is a benzyl, alkylbenzyl, hydroxybenzyl, alkoxycarbonylbenzyl, aryloxycarbonylbenzyl, carboxybenzyl, nitrobenzyl, cyanobenzyl, or halobenzyl group.

5. The compound of claim 1, wherein the aryl group is a phenyl, naphthyl, anthracenyl, pyridinyl, indolyl, furanyl, alkylphenyl, hydroxyphenyl, alkoxycarbonylphenyl, aryloxycarbonylphenyl, nitrophenyl, cyanophenyl, halophenyl group, mercaptophenyl, or aminophenyl group.

6. A compound of claim 1 selected from the group consisting of:

N-(2-n-propylpentanoyl)glycinamide;

N-(2-n-propylpentanoyl)glycine-N'-methylamide;

N-(2-n-propylpentanoyl)glycine-N'-butylamide;

N-(2-n-propylpentanoyl)leucinamide;

N-(2-n-propylpentanoyl)alanine-N'-benzylamide;

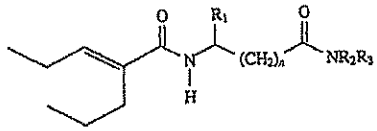
N-(2-n-propylpentanoyl)alapinamide;

N-(2-n-propylpentanoyl)-2-phenylglycinamide;

N-(2-n-propylpentanoyl)threoninamide; and

N-(2-n-propylpentanoyl)glycine-N',N'-dimethylamide.

7. A compound having the structure:



wherein R_1, R_2, and R_3 are independently the same or different and are hydrogen, a C_1–C_6 alkyl group, an aralkyl group, or an aryl group, and n is an integer which is greater than or equal to 0 and less than or equal to 3.

8. The compound of claim 7, wherein the C_1–C_6 alkyl group is a linear chain alkyl group.

9. The compound of claim 7, wherein the C_1–C_6 alkyl group is a branched chain alkyl group.

10. The compound of claim 7, wherein the aralkyl group is a benzyl, alkylbenzyl, hydroxybenzyl, alkoxycarbonylbenzyl, aryloxycarbonylbenzyl, carboxybenzyl, nitrobenzyl, cyanobenzyl, or halobenzyl group.

11. The compound of claim 7, wherein the aryl group is a phenyl, naphthyl, anthracenyl, pyridinyl, indolyl, furanyl, alkylphenyl, hydroxyphenyl, alkoxycarbonylphenyl, aryloxycarbonylphenyl, nitrophenyl, cyanophenyl, halophenyl group, mercaptophenyl, or aminophenyl group.

12. A compound of claim 7 selected from the group consisting of:

N-(2-n-propylpent-2-enoyl)glycinamide;

N-(2-n-propylpent-2-enoyl)alaninamide; and

N-(2-n-propylpent-2-enoyl)glycine-N'-methylamide.

13. A pharmaceutical composition which comprises the compound of claims 1 or 7 or a in a therapeutically effective amount and a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13 wherein the therapeutically effective amount is an amount from about 10 to about 500 mg.

15. The pharmaceutical composition of claim 14, wherein the carrier is a solid and the composition is a tablet.

16. The pharmaceutical composition of claim 14, wherein the carrier is a gel and the composition is a suppository.

17. The pharmaceutical composition of claim 14, wherein the carrier is a liquid and the composition is a solution.

18. A method of treating a subject afflicted with epilepsy which comprises administering to the subject an amount of the compound of claim 7 effective to treat epilepsy in the subject.

19. A method of treating a subject afflicted with epilepsy which comprises administering to the subject an amount of the compound of claim 1 effective to treat epilepsy in the subject.

* * * * *

ATTACHMENT D

United States Patent [19]

Roth

[11] **Patent Number: 4,681,893**

[45] **Date of Patent: Jul. 21, 1987**

[54] **TRANS-6-[2-(3- OR 4-CARBOXAMIDO-SUBSTITUTED PYRROL-1-YL)ALKYL]-4-HYDROXYPYRAN-2-ONE INHIBITORS OF CHOLESTEROL SYNTHESIS**

[75] Inventor: Bruce D. Roth, Ann Arbor, Mich.

[73] Assignee: Warner-Lambert Company, Morris Plains, N.J.

[21] Appl. No.: 868,867

[22] Filed: May 30, 1986

[51] Int. Cl.[4] A61K 31/40; A61K 31/35; C07D 207/327

[52] U.S. Cl. 514/422; 514/423; 546/256; 546/275; 548/517; 548/537

[58] Field of Search 548/517, 537; 514/422, 514/423

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Primary Examiner—Joseph Paul Brust

Attorney, Agent, or Firm—Jerry F. Janssen

[57] ABSTRACT

Certain trans-6-[2-(3- or 4-carboxamido-substituted pyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones and the corresponding ring-opened acids derived therefrom which are potent inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase and are thus useful hypolipidemic or hypocholesterolemic agents. Pharmaceutical compositions containing such compounds, and a method of inhibiting the biosynthesis of cholesterol employing such pharmaceutical compositions are also disclosed.

9 Claims, No Drawings

TRANS-6-[2-(3- OR 4-CARBOXAMIDO-SUBSTITUTED PYRROL-1-YL)ALKYL]-4-HYDROXYPYRAN-2-ONE INHIBITORS OF CHOLESTEROL SYNTHESIS

BACKGROUND OF THE INVENTION

The present invention is related to compounds and pharmaceutical compositions useful as hypocholesterolemic and hypolipidemic agents. More particularly, this invention concerns certain trans-6-[2-(3- or 4-carboxamidosubstitutedpyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones and the corresponding ring-opened acids derived therefrom which are potent inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase), pharmaceutical compositions containing such compounds, and a method of inhibiting the biosynthesis of cholesterol employing such pharmaceutical compositions.

High levels of blood cholesterol and blood lipids are conditions involved in the onset of arteriosclerosis. It is well known that inhibitors of HMG-CoA reductase are effective in lowering the level of blood plasma cholesterol, especially low density lipoprotein cholesterol (LDL-C), in man (cf. M. S. Brown and J. L. Goldstein, *New England Journal of Medicine*, 305, No. 9, 515–517 (1981). It has now been established that lowering LDL-C levels affords protection from coronary heart disease (cf. *Journal of the American Medical Association*, 251, No. 3, 351–374 (1984).

Moreover, it is known that certain derivatives of mevalonic acid (3,5-dihydroxy-3-methylpentanoic acid) and the corresponding ring-closed lactone form, mevalonolactone, inhibit the biosynthesis of cholesterol (cf. F. M. Singer et al., *Proc. Soc. Exper. Biol. Med.*, 102: 370 (1959) and F. H. Hulcher, *Arch. Biochem. Biophys.*, 146: 422 (1971)).

U.S. Pat. Nos. 3,983,140; 4,049,495 and 4,137,322 disclose the fermentative production of a natural product, now called compactin, having an inhibitory effect on cholesterol biosynthesis. Compactin has been shown to have a complex structure which includes a mevalonolactone moiety (Brown et al., *J. Chem. Soc. Perkin* I (1976) 1165.

U.S. Pat. No. 4,255,444 to Oka et al. discloses several synthetic derivatives of mevalonolactone having antilipidemic activity.

U.S. Pat. Nos. 4,198,425 and 4,262,013 to Mitsue et al. disclose aralkyl derivatives of mevalonolactone which are useful in the treatment of hyperlipidemia.

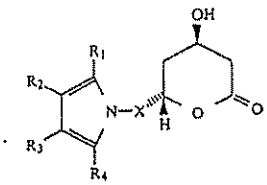
U.S. Pat. no. 4,375,475 to Willard et al. discloses certain substituted 4-hydroxytetrahydropyran-2-ones which, in the 4(R)-trans-stereoisomeric form, are inhibitors of cholesterol biosynthesis.

Published PCT application No. WO 84/01231 discloses certain indole analogs and derivatives of mevalonolactone having utility as hypolipoproteinemic and antiatherosclerotic agents.

SUMMARY OF THE INVENTION

In accordance with the present invention, there are provided certain trans-6-[2-(3- or 4-carboxamido-substituted pyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones and the corresponding ring-opened hydroxy-acids derived therefrom which are potent inhibitors of cholesterol biosynthesis by virtue of their ability to inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase).

In particular, in its broadest aspect the present invention provides compounds of structural formula I



I

wherein X is $—CH_2—$, $—CH_2CH_2—$, $—CH_2CH_2CH_2—$ or $—CH_2CH(CH_3)—$.

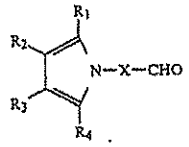
R_1 is 1-naphthyl; 2-naphthyl; cyclohexyl; norbornenyl; 2-, 3-, or 4-pyridinyl; phenyl, phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

Either R_2 or R_3 is $—CONR_5R_6$ where R_5 and R_6 are independently hydrogen; alkyl of from one to six carbon atoms; 2-, 3-, or 4-pyridinyl; phenyl; phenyl substituted with fluorine, chlorine, bromine, cyano, trifluoromethyl, or carboalkoxy of from three to eight carbon atoms; and the other of R_2 or R_3 is hydrogen; alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; phenyl; or phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

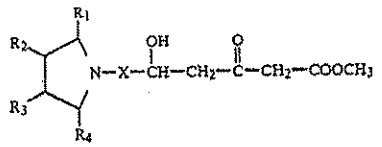
R_4 is alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; or trifluoromethyl.

Also contemplated as falling within the scope of the present invention are the hydroxy acids, and pharmaceutically acceptable salts thereof, derived from the opening of the lactone ring of the compounds of structural formula I above.

In another aspect of the present invention, there is provided a method of preparing the compounds of structural formula I above which comprises the steps of (a) first reacting a substituted [(pyrrol-1-yl)alkyl]aldehyde compound of the formula

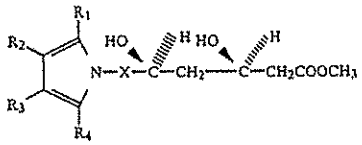


with the dilithio or sodio-lithio salt of methyl acetoacetate to form a compound of the structure



(b) reducing the product of step (a) with a trialkylborane compound such as tributylborane in the presence of sodium borohydride in an inert solvent;

(c) oxidizing the product of step (b) with alkaline aqueous hydrogen peroxide solution to produce a compound of the formula



and

(d) cyclizing the product step (c) to a lactone of formula I above by heating in an inert solvent such as toluene or, alternatively converting the product of step (c) to a pharmaceutically acceptable salt by conventional methods.

In yet another aspect, the present invention provides pharmaceutical compositions useful as hypolipidemic or hypocholesterolemic agents comprising a hypolipidemic or hypocholesterolemic effective amount of a compound in accordance with this invention as set forth above, in combination with a pharmaceutically acceptable carrier.

In another aspect, the present invention provides a method of inhibiting cholesterol biosynthesis in a patient in need of such treatment by administering an effective amount of a pharmaceutical composition as defined above.

DETAILED DESCRIPTION

The compounds of the present invention comprise a class of trans-6-[2-(3- or 4-carboxamidosubstituted pyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones in which the pyran-2-one moiety is attached, through an alkyl chain, to the substituted pyrrole nucleus at the nitrogen, or 1-position, of the pyrrole. The alkyl group may be methylene, ethylene, propylene, or methylethylene. The preferred alkyl chain linking the substituted pyrrole nucleus and the 4-hydroxypyran-2-one ring is ethylene.

The compounds of structural formula I above possess two asymmetric carbon centers, one at the 4-hydroxy position of the pyran-2-one ring, and the other at the 6-position of the pyran-2-one ring where the alkylpyrrole group is attached. This asymmetry gives rise to four possible isomers, two of which are the R-cis- and S-cis-isomers and the other two of which are the R-trans- and S-trans-isomers. This invention contemplates only the trans- form of the compounds of formula I above.

In the compounds of the present invention, position 2 of the substituted pyrrole nucleus is substituted with 1-naphthyl; 2-naphthyl; cyclohexyl; norbornenyl; 2-, 3-, or 4-pyridinyl; phenyl, phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms. Preferred substituent groups at the 2-position of the pyrrole nucleus are phenyl and substituted phenyl.

In the compounds of this invention, position 5 of the pyrrole nucleus is substituted with alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; or trifluoromethyl. Preferred substituents are alkyl or trifluoromethyl with isopropyl being particularly preferred.

The preferred reaction sequence which is used to prepare compounds of the present invention involves the cycloaddition of a disubstituted acetylene, in which one substituent is carboxamido or N-substituted carboxamido, to an appropriately substituted N-acylaminocarboxylic acid to form a substituted pyrrole. This addition may occur in either of two ways, leading to a substituted pyrrole addition product in which the carboxamido substituent resides on either carbon 3 or 4 of the pyrrole nucleus.

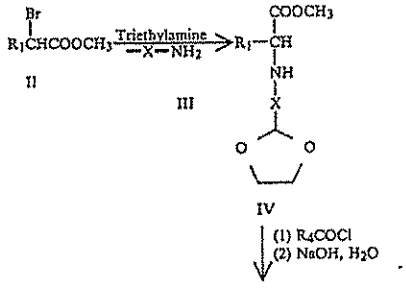
Thus, in compounds of the present invention, the substituent at either position 3 or 4 of the pyrrole nucleus is $-CONR_5R_6$ where R_5 and R_6 are independently hydrogen; alkyl of from one to six carbon atoms; 2-, 3-, or 4-pyridinyl; phenyl; phenyl substituted with fluorine, chlorine, bromine, cyano, trifluoromethyl, or carboalkoxy of from three to eight carbon atoms and the other of the two positions is unsubstituted or is substituted with alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; phenyl; or phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

Preferred groups for R_5 and R_6 are hydrogen, phenyl, or substituted phenyl. In a particularly preferred group of compounds within the present invention, R_5 is hydrogen and R_6 is phenyl or substituted phenyl.

The compounds of this invention are prepared by the general reaction scheme outlined in Reaction Sequence 1 which takes advantage of the chemistry of mesionic compounds of the type described originally by R. Huisgen et al., *Ang. Chem. Int. Ed.*, 3: 136 (1964).

The known, or readily prepared, α-haloesters of structural formula II are reacted with the known 2-[1-(2-aminoalkyl)]-1,3-dioxalane, III, in the presence of an acid scavenger such as triethylamine to produce the N-alkyl-α-aminoesters, IV. The aminoesters, IV are

REACTION SEQUENCE I



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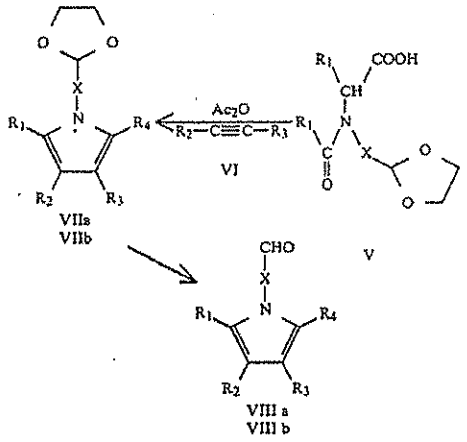
REACTION SEQUENCE I



VIIa
VIIb

VI

V

VIII a
VIII b

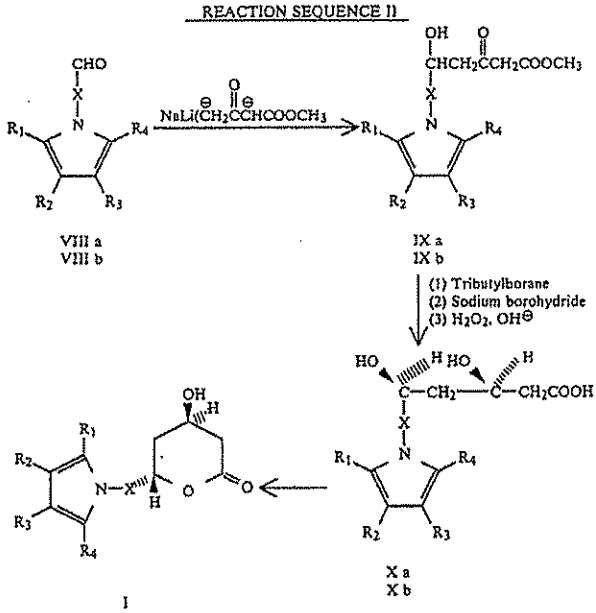
known in the art, and subsequently further purified, if desired, by recrystallization. On the other hand, in the case where R_4 is 1-methylethyl, the cyclo-addition reaction yields predominantly one product which can be purified by recrystallization alone.

Hydrolysis of the acetal function of compounds VIIa and VIIb in aqueous acid solution affords the aldehydes VIIIa and VIIIb. The aldehydes, VIII, are further converted to compounds of the present invention by the processes depicted in Reaction Sequence 2.

The aldehyde compounds, VIII, are reacted with the dilithium or lithio-sodio salt of methyl acetoacetate to produce the corresponding 7-(substituted-pyrrolyl)-5-hydroxy-3-oxoheptanoates, IX. The heptanoates, IX, are dissolved in a polar solvent such as tetrahydrofuran, through which a small amount of air has been bubbled. A slight excess of a trialkylborane, such as tributylborane, is added to the mixture which is then cooled to a temperature of preferably between about 0° C. and −78° C. after which sodium borohydride is added.

The mixture is stirred for about one to two hours and then oxidized by the addition of basic aqueous hydrogen peroxide solution. The reaction produces the 7-(substituted-pyrrolyl)-3,5-dihydroxyheptanoic acids,

REACTION SEQUENCE II



VIII a
VIII b

IX a
IX b

(1) Tributylborane
(2) Sodium borohydride
(3) H_2O_2, $OH^{\ominus}$

X a
X b

I

acylated with an acid halide and subsequently hydrolyzed in aqueous base solution to produce the N-acyl-N-alkyl aminoacids, V.

The N-acyl-N-alkyl aminoacids, V, are reacted with the appropriately substituted carboxamido acetylenic compounds, VI, in the presence of an acid anhydride to produce a mixture of the isomeric substituted pyrrole compounds VIIa and VIIb. Depending upon the substituents present, this cyclo-addition reaction affords differing ratios of the two products. For example, in the situation where R_4 is trifluoromethyl, the reaction yields roughly equimolar amounts of the two isomeric products. In such situations, the two isomeric products are separated by chromatographic techniques well

X, in which the product contains a predominance of the desired R*,R* configuration at carbon atoms three and five which bear the hydroxy groups.

The acids may be converted to a corresponding pharmaceutically acceptable salt by conventional means, if desired, or cyclized to the trans-6-[2-(substituted-pyrrol-1-yl)alkyl]pyran-2-ones, I, by dehydration in an inert solvent such as refluxing toluene with azeotropic removal of water. This cyclization step has been found to produce material containing from 85–90% of the desired trans-configuration of the 4-hydroxy group relative to the 6-(substituted-pyrrol-1-yl)alkyl group on the pyran-2-one lactone ring.

The ring-opened hydroxy acids of structural formula II above are intermediates in the synthesis of the lactone compounds of formula I and may be used in their free acid form or in the form of a pharmaceutically acceptable metal or amine salt in the pharmaceutical method of the present invention. These acids react to form pharmaceutically acceptable metal and amine salts. The term "pharmaceutically acceptable metal salt" contemplates salts formed with the sodium, potassium, calcium, magnesium, aluminum, iron, and zinc ions. The term "pharmaceutically acceptable amine salt" contemplates salts with ammonia and organic nitrogenous bases strong enough to form salts with carboxylic acids. Bases useful for the formation of pharmaceutically acceptable nontoxic base addition salts of compounds of the present invention form a class whose limits are readily understood by those skilled in the art.

The free acid form of compounds of the present invention may be regenerated from the salt form, if desired, by contacting the salt with a dilute aqueous solution of an acid such as hydrochloric acid.

The base addition salts may differ from the free acid forms of the compounds of this invention in such physical characteristics as solubility and melting point, but are otherwise considered equivalent to the free acid form for the purposes of this invention.

The compounds of the present invention may exist in solvated or unsolvated form. In general, the solvated forms with pharmaceutically acceptable solvents such as water, ethanol and the like, are equivalent to the unsolvated forms for the purposes of this invention.

The compounds of this invention are useful as hypocholesterolemic or hypolipidemic agents by virtue of their ability to inhibit the biosynthesis of cholesterol through inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase).

The ability of compounds of the present invention to inhibit the biosynthesis of cholesterol was measured by two methods. A first method (designated CSI screen) utilized the procedure described by R. E. Dugan et al., *Archiv. Biochem. Biophys.*, (1972), 152, 21–27. In this method, the level of HMG-CoA enzyme activity in standard laboratory rats is increased by feeding the rats a chow diet containing 5% cholestyramine for four days, after which the rats are sacrificed.

The rat livers are homogenized, and the incorporation of cholesterol-^{14}C-acetate into nonsaponifiable lipid by the rat liver homogenate is measured. The micromolar concentration of compound required for 50% inhibition of sterol synthesis over a one-hour period is measured, and expressed as an IC_{50} value.

A second method (designated COR screen) employed the procedure detailed by T. Kita, et al., *J. Clin. Invest.*, (1980), 66: 1094–1100. In this method, the amount of ^{14}C-HMG-CoA converted to ^{14}C-mevalonate in the presence of a purified enzyme preparation of HMG-CoA reductase was measured. The micromolar concentration of compound required for 50% inhibition of cholesterol synthesis was measured and recorded as an IC_{50} value.

The activity of several representative examples of compounds in accordance with the present invention appears in Table 1, and is compared with that of the prior art compound, compactin.

For preparing pharmaceutical compositions from the compounds of this invention, inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersable granules, capsules, cachets, and suppositories.

A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

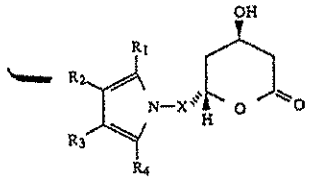
In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component. In tablets, the active compound is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

For preparing suppositories, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted, and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient sized molds and allowed to cool and solidify.

Powders and tablets preferably contain between about 5 to about 70% by weight of the active ingredient. Suitable carriers are magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is

TABLE 1



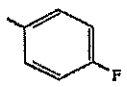
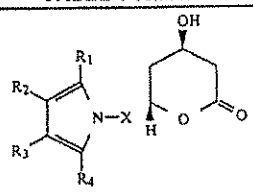
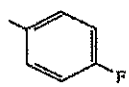
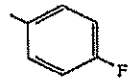
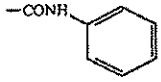
Compound	X	R_1	R_2	R_3	R_4	IC_{50} (Micromoles/liter) CSI	COR
1	—CH_2CH_2—			—CONH— 	—$CH(CH_3)_2$	0.035	0.050

TABLE 1-continued



Compound	X	R_1	R_2	R_3	R_4	IC_{50} (Micromoles/liter) CSI	COR
2	$-CH_2CH_2-$		$-CONH-$ 		$-CF_3$	0.40	0.40
3	$-CH_2CH_2-$			$-CONH-$ 	$-CF_3$	0.018	0.020
Compactin (Prior art)						0.026	0.028

surrounded by a carrier, which is thus in association with it. In a similar manner, cachets are also included. Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions suitable for oral or parenteral administration, or suspensions and emulsions suitable for oral administration. Sterile water solutions of the active component or sterile solutions of the active component in solvents comprising water, ethanol, or propylene glycol may be mentioned as examples of liquid preparations suitable for parenteral administration.

Sterile solutions may be prepared by dissolving the active component in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile compound in a previously sterilized solvent under sterile conditions.

Aqueous solutions for oral administration can be prepared by dissolving the active compound in water and adding suitable flavorants, coloring agents, stabilizers, and thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water together with a viscous material such as natural or synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other suspending agents known to the pharmaceutical formulation art.

Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms.

In therapeutic use as hypolipidemic or hypocholesterolemic agents, the compounds utilized in the pharmaceutical method of this invention are administered to the patient at dosage levels of from 40 mg to 600 mg per day. For a normal human adult of approximately 70 kg or body weight, this translates to a dosage of from about 0.5 mg/kg to about 8.0 mg/kg of body weight per day.

The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed. Determination of optimum dosages for a particular situation is within the skill of the art.

The following examples illustrate particular methods for preparing compounds in accordance with this invention. These examples are illustrative and are not to be read as limiting the scope of the invention as it is defined by the appended claims.

EXAMPLE 1

Preparation of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo2H-pyran-2-yl)ethyl]-pyrrole-3-carboxamide

Step A: Preparation of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester

A solution of 26 g (220 mmol) of 2-[1-(2-aminoethyl)]-1,3-dioxalane in 50 ml of acetonitrile was added at room temperature with stirring to a solution of 200 mmol of α-bromo-4-fluorobenzeneacetic acid, ethyl ester (J. W. Epstein et al., *J. Med. Chem.*, 24: 481–490 (1981)) and 42 ml (300 mmol) of triethylamine in 350 ml of acetonitrile. The resulting mixture was stirred at room temperature overnight and then poured into 500 ml of diethyl ether. The resulting suspension was extracted with 300 ml of water and then twice with 300-ml portions of 2M hydrochloric acid. The combined extracts were made basic with 25% aqueous sodium hydroxide solution and extracted twice with 500-ml portions of ethyl acetate. The ethyl acetate extracts were combined, washed successively with water and brine, and then dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the residue concentrated to yield 49.5 g of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester.

The 90 MHz proton magnetic resonance spectrum of the product in deuterochloroform exhibited signals at 1.18 (triplet, 3H, J=7 Hz); 1.85 (multiplet, 2H); 2.20

(broad singlet, 1H); 2.6 (multiplet, 2H); 3.85 (multiplet, 4H); 4.1 (quartet, 2H, J=7 Hz); 4.22 (singlet, 1H); 4.83 (triplet, 1H, J=4.5 Hz); and 6.8–7.3 (multiplet, 4H) parts per million downfield from tetramethylsilane.

Step B. Preparation of α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid, ethyl ester.

Thirty grams (100 mmol) of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester from Step A were dissolved in 200 ml of dichloromethane together with 28.6 ml (205 mmol) of triethylamine and the resulting mixture was cooled to 0° C. under dry nitrogen. A solution of 11 ml (105 mmol) of isobutyryl chloride in 50 ml of dichloromethane was slowly added with stirring. After addition was complete, the mixture was stirred for an additional 60 minutes and then poured into 100 ml of diethyl ether. The ether solution was washed successively with portions of water, 2M hydrochloric acid, sodium bicarbonate solution, and brine, and then dried over anhydrous magnesium sulfate. Evaporation of the solvents yielded 35 g of α-[[2-(1,3-dioxolan-2-yl)-ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzene-acetic acid, ethyl ester.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of the product exhibited signals at 1.2 (multiplet, 9H); 1.7 (multiplet, 2H); 2.85 (multiplet, 1H); 3.35 (multiplet, 2H); 3.80 (multiplet, 4H); 4.20 (quartet, 2H, J=7 Hz); 4.60 (triplet, 1H, J=4.5 Hz); 5.81 (singlet, 1H); and 6.8–7.3 (multiplet, 4H) parts per million downfield from tetramethylsilane.

Step C. Preparation of α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid

A solution of 35 g (95.3 mmol) of the ester from Step B and 12 g (300 mmol) of sodium hydroxide in 480 ml of 5:1 methanol water was heated under reflux and stirred for two hours. The solution was cooled to room temperature, concentrated, and diluted by the addition of 500 ml of water. The resulting solution was extracted with ether and the aqueous layer was acidified with ice-cold 6M hydrochloric acid and then extracted twice with 300-ml portions of ethyl acetate.

The combined extracts were washed with brine, dried over anhydrous magnesium sulfate, and evaporated to yield 30 g of crude α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid which was used without further purification.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of the product exhibited signals at 1.11 (doublet, 6H, J=7 Hz); 1.4–1.9 (multiplet, 2H); 2.85 (multiplet, 1H); 3.32 (multiplet, 2H); 3.75 (multiplet, 4H); 4.52 (triplet, 1H, J=4.5 Hz); 5.73 (singlet, 1H); and 6.8–7.3 (multiplet, 4H) parts per million downfield from tetramethylsilane.

Step D. Preparation of N,3-diphenylpropynamide

A solution of 171 mmol of dicyclohexylcarbodiimide in 250 ml of dichloromethane was added dropwise over a two hour period at 0° C. to a suspension of 171 mmol of propiolic acid, 179.6 mmol of aniline, and 5 mmol of 4-dimethylaminopyridine in 400 ml of dichloromethane. After addition was complete, the mixture was stirred for an additional 30 minutes and then diluted with diethyl ether. The resulting mixture was filtered through silica gel, concentrated, and the residue recrystallized to provide 30.5 g of N,3-diphenyl-2-propynamide, mp 122°–123° C.

Analyzed for $C_{15}H_{13}NO$: Calc.: C, 80.69%; H, 5.87%; N, 6.27%; Found: C, 80.54%; H, 5.58%; N, 6.52%.

The infrared spectrum of a KBr pellet of the compound showed principal peaks at 2215, 1630, 1595,1549, 1490, 1445, 1330, 756, and 691 reciprocal centimeters.

Step E. Preparation of 1-[2-(1,3-dioxalan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide

A solution of 95 g (280 mmol) of α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid, prepared as described in Step C above, and 98 g (439 mmol) of N,2-diphenylpropenoic carboxamide, prepared as described in Step D above, was heated at 90° C. with stirring for four hours, (Vigorous gas evolution occurred for two hours.) After this time, the mixture was cooled to room temperature and chromatographed twice on silica gel, eluting with 4:1 hexane:ethyl acetate to separate the product (R_f=0.35) from the starting material (R_f=0.5).

Recrystallization of the product from isopropyl ether provided 59.5 g (119.3 mmol) of 1-[2-(1,3-dioxalan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide, mp 159°–162° C.

Analyzed for $C_{31}H_{31}FN_2O_3$: Calc.: C, 74.68%; H, 6.27%; N, 5.62%; Found: C, 75.04%; H, 6.12%; N, 5.89%.

Step F. Preparation of 5-(4-fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide

A solution of 59 g (118.3 mmol) of 1-[2-(1,3-dioxalan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide, from Step E above, and 0.4 ml of concentrated hydrochloric acid in 1200 ml of anhydrous ethanol was heated under reflux with stirring for 24 hours. After this time the mixture was cooled to room temperature, concentrated, and the residue taken up in 1200 ml of 3:1 acetone:water and 5 g of p-toluenesulfonic acid was added. This mixture was heated under reflux with stirring for two days after which time the solution was cooled to room temperature and partitioned between 1 liter of diethyl ether and 200 ml of brine solution.

The organic phase was separated, washed successively with sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated. The oil which resulted was dissolved in the minimum amount required of hot isopropyl ether. The crystals which formed upon cooling were collected by filtration to yiled 36.8 g of 5-(4-fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide. A further crop of 9.8 g of crystals were obtained from the mother liquor.

Analyzed for $C_{29}H_{27}FN_2O_3$: Calc.: C, 76.63%; H, 5.99%; N, 6.16%; Found: C, 76.48%; H, 6.20%; N, 6.14%.

Step G. Preparation of 2-(4-fluorophenyl)-δ-hydroxy-5-(1-methylethyl)-β-oxo-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, methyl ester

A solution of methyl acetoacetate (26.4 ml, 243 mmol) in 250 ml of anhydrous tetrahydrofuran was added dropwise to a stirred suspension of hexane-washed sodium hydride (6.4 g, 267 mmol) in 200 ml of tetrahydrofuran at 0° C. When gas evolution was complete, 97.2 ml of 2.5M n-butyl lithium was added dropwise over a period of 60 minutes.

The resulting solution was stirred for 30 minutes at 0° C. and then cooled to −78° C. after which a solution of

36.8 g (80.9 mmol) of 5-(4-fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide, from Step F above, in 100 ml of tetrahydrofuran was added over a period of thirty minutes. The resulting solution was stirred for 30 minutes at −78° C. and then warmed to 0° C. where it was held for an additional 60 minutes.

The mixture was then acidified by the dropwise addition of 300 ml of ice-cold 3M hydrochloric acid, diluted with ether, washed successively with water and brine, dried over anhydrous magnesium sulfate, and concentrated. Flash chromatography of the residue yielded 37.9 g of 2-(4-fluorophenyl)-δ-hydroxy-5-(1-methylethyl)-β-oxo-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, methyl ester.

The 90 MHz proton magnetic resonance spectrum of the product exhibited signals at 1.50 (doublet, 6H, J=7 Hz); 1.8 (multiplet, 2H); 2.45 (doublet, 2H, J=7 Hz); 2.8 (broad, 1H); 3.33 (singlet, 2H); 3.5 (multiplet, 1H); 3.67 (singlet, 3H); 3.8–4.0 (multiplet, 2H); and 6.8–7.3 (multiplet, 14H) parts per million downfield from tetramethylsilane.

Step H. Preparation of R*,R*-2-(4-fluorophenyl-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid and trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide

Air (60 ml) was bubbled via a syringe through a solution of 2-(4-fluorophenyl)-δ-hydroxy-5-(1-methylethyl)-β-oxo-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, methyl ester (48 g, 84.1 mmol) and 92.5 ml of 1M tributylborane in 100 ml of anhydrous tetrahydrofuran. The mixture was stirred overnight at room temperature and then cooled to −78° C. Sodium borohydride (3.85 g, 101.8 mmol) was added to the cooled mixture in one portion. The mixture was allowed to warm slowly to 0° C. over a period of three hours, during which there was vigorous gas evolution.

The dry ice-acetone bath applied to the reaction vessel was replaced by an ice bath and 18.3 ml of glacial acetic acid were added dropwise, followed by 204 ml of 3M aqueous sodium hydroxide solution and 30.5 ml of 30% aqueous hydrogen peroxide solution.

The mixture was vigorously stirred while being allowed to warm to room temperature overnight. The mixture was then partitioned between diethyl ether and water and the aqueous layer was separated, acidified, and extracted with ethyl acetate.

The ethyl acetate extract was washed with brine, dried, and evaporated to yield crude R*,R*-2-(4-fluorophenyl-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid which was used without further purification.

The crude acid was taken up in toluene and lactonized by heating under reflux for six hours. This mixture was chromatographed to provide 30 g of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide as a foamy solid, mp 90°–97° C.

Analyzed for $C_{33}H_{33}FN_2O_4$: Calc.: C, 73.31%; H, 6.15%; N, 5.18%; Found: C, 73.46%; H, 6.31%; N, 5.28%.

This material was found by HPLC analysis to comprise a 9:1 molar ratio of the cis- and trans-isomeric forms of the product. Recrystallization from toluene-ethyl acetate yield the essentially pure trans-form, mp 148°–149° C.

EXAMPLE 2

Preparation of R*,R*-2-(4-fluoro-phenyl-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, sodium salt

A mixture of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide (10 g, 18.5 mmol) and 0.74 g (18.5 mmol) of sodium hydroxide in 90 ml of a 1:2 mixture of tetrahydrofuran-water was cooled to 0° C. This mixture was allowed to warm slowly to 25° C.; after which time it was concentrated and the residual solid dried under vacuum.

The infrared spectrum of the product exhibited principal absorption peaks at 3400, 1651, 1598, 1565, 1511, 1438, 1412, 1316, 1224, 1159, 844, 754, and 702 reciprocal centimeters.

The 90 MHz proton magnetic resonance spectrum of a hexadeutero dimethylsulfoxide solution of the product exhibited signals at 1.34 (doublet, J=7 Hz, 6H); 1.5 (multiplet, 4H); 1.80 (doublet of doublets, J=15, 8 Hz, 1H); 1.99 (doublet of doublets, J=15, 4 Hz, 1H); 3–4 (multiplet, 8H); 6.9–7.3 (multiplet, 12H); 7.50 (doublet, J=8 Hz, 2H); and 9.85 (singlet, 1H) parts per million downfield from tetramethylsilane.

EXAMPLES 3 AND 4

Preparation of trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-(trifluoromethyl)-pyrrole-3-carboxamide and trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-(trifluoromethyl)pyrrole-3-carboxamide

Step A. Preparation of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid.

α-[[2-(1,3-Dioxolan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester (36.5 g, 122.8 mmol, prepared as described above in Example 1, Step A) was dissolved in 1500 ml of a 5:1 mixture of methanol-water together with 7.6 g of sodium hydroxide. This mixture was heated under reflux for a period of two and one-half hours after which time the solvents were removed under vacuum.

The solid residue was taken up in 325 ml of water and a mixture of 14 ml of glacial acetic in 28 ml of water was added with stirring. After stirring for a time, an additional 3 ml of glacial acetic acid were added and the mixture was chilled for 75 minutes. The solids were collected by filtration, washed with water and then ethyl acetate and dried to yield α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, mp 218°–220° C.

Step B. Preparation of a mixture of 5-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-2-(trifluoromethyl)-1H-pyrrole-3-carboxamide and 2-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-5-(trifluoromethyl)-1H-pyrrole-3-carboxamide

α-[[2-(1,3-Dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid (6.06 g, 22.5 mmol) was dissolved in 45 ml of trifluoroacetic anhydride and 7.47 g (33.8 mmol) of N,3-diphenyl-2-propynamide (prepared as described above in Example 1, Step D) was added. The resulting mixture was heated under reflux for a period of five and one-half hours. The mixture was then cooled, and 1.74

ml of trifluoroacetic acid were added and the mixture was stirred overnight.

The excess trifluoroacetic anhydride was removed under vacuum, and water was added, followed by sufficient acetone to give a homogenous solution. This solution was stirred at room temperature for three hours. The mixture was seeded with N,3-diphenyl-2-propynamide, and a precipitate formed. After three hours, this precipitate was removed by filtration.

The acetone was removed from the filtrate under vacuum and the solid residue was taken up in ether, washed successively with two portions of water, two portions of sodium bicarbonate solution, and two portions of brine and dried over anhydrous magnesium sulfate. The ether was removed under vacuum to yield a crude mixture of the two title compounds.

This mixture was separated by column chromatography on 600 g of silica gel, eluting with a 4:1 mixture of hexane-ethyl acetate.

The first fraction eluted was 5-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-2-(trifluoromethyl)-1H-pyrrole-3-carboxamide.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of this material exhibited signals at 2.73 (triplet, J=7 Hz, 2H); 4.21 (triplet, J=7 Hz, 2H); 6.7–7.3 (multiplet, 5H); 7.40 (singlet, 5H), and 9.43 (singlet, 1H) parts per million downfield from tetramethylsilane.

The second compound eluted from the column was 2-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-5-(trifluoromethyl)-1H-pyrrole-3-carboxamide.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of this material exhibited signals at 2.67 (triplet, J=7 Hz, 2H); 4.25 (triplet, J=7 Hz, 2H); 7.0–7.3 (multiplet, 14H); and 9.43 (singlet, 1H) parts per million downfield from tetramethylsilane.

Step C. Preparation of trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-(trifluoromethyl)-pyrrole-3-carboxamide and trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-(trifluoromethyl)-pyrrole-3-carboxamide

Employing the general methods detailed in Example 1, Steps G and H, the title compounds were prepared from the aldehyde compounds of this example, Step B.

The elemental analyses of the two title compounds were:

For trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-(trifluoromethyl)-pyrrole-3-carboxamide:

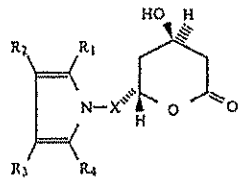
Analyzed for $C_{31}H_{26}N_2O_4$: Calc.: C, 65.72%; H, 4.63%; N, 4.94%; Found: C, 65.82%; H, 4.91%; N, 4.69%.

The trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-(trifluoromethyl)-pyrrole-3-carboxamide was found, upon recrystallization from toluene to contain 0.25 mols of toluene as solvent of crystallization, mp 106°–111° C.

Analyzed for $C_{31}H_{26}N_2O_4 \cdot 0.25C_7H_8$: Calc.: C, 66.72%; H, 4.79%; N, 4.72%; Found: C, 66.81%; H, 4.86%; N, 4.60%.

I claim:

1. A compound of structural formula I



wherein

X is $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, or $-CH_2CH(CH_3)-$;

R_1 is

1-naphthyl;
2-naphthyl;
cyclohexyl;
norbornenyl;
phenyl;
phenyl substituted with
fluorine,
chlorine,
bromine,
hydroxyl,
trifluoromethyl,
alkyl of from one to four carbon atoms,
alkoxy of from one to four carbon atoms, or
alkanoyloxy of from two to eight carbon atoms;

either of R_2 or R_3 is $-CONR_5R_6$ where R_5 and R_6 are independently

hydrogen;
alkyl of from one to six carbon atoms;
phenyl;
phenyl substituted with
fluorine,
chlorine,
bromine,
cyano,
trifluoromethyl, or
carboalkoxy of from three to eight carbon atoms;

and the other of R_2 or R_3 is

hydrogen;
alkyl of from one to six carbon atoms;
cyclopropyl;
cyclobutyl;
cyclopentyl;
cyclohexyl;
phenyl; or
phenyl substituted with
fluorine,
chlorine,
bromine,
hydroxyl,
trifluoromethyl,
alkyl of from one to four carbon atoms,
alkoxy of from one to four carbon atoms, or
alkanoyloxy of from two to eight carbon atoms;

R_4 is

alkyl of from one to six carbon atoms;
cyclopropyl;
cyclobutyl;
cyclopentyl;
cyclohexyl; or
trifluoromethyl;

or a hydroxy acid or pharmaceutically acceptable salts thereof, corresponding to the opened lactone

ring of the compounds of structural formula I above.

2. A compound as defined by claim 1 wherein X is $-CH_2CH_2-$.

3. A compound as defined by claim 2 wherein R_1 is phenyl; or phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

4. A compound as defined by claim 2 wherein R_4 is alkyl of from one to six carbon atoms.

5. A compound as defined by claim 1 having the name trans-(±)-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide.

6. A compound as defined by claim 1 having the name trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-trifluoromethyl-1H-pyrrole-3-carboxamide.

7. A compound as defined by claim 1 having the name trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-trifluoromethyl-1H-pyrrole-3-carboxamide.

8. A pharmaceutical composition, useful as a hypocholesterolemic agent, comprising a hypocholesterolemic effective amount of a compound in accordance with claim 1 in combination with a pharmaceutically acceptable carrier.

9. A method of inhibiting cholesterol biosynthesis in a patient in need of such treatment by administering a pharmaceutical composition as defined by claim 8.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE EXTENDING PATENT TERM UNDER 35 U.S.C. § 156

PATENT NO.	:	4,681,893
ISSUED	:	July 21, 1987
INVENTOR(S)	:	Bruce D. Roth
PATENT OWNER	:	Warner-Lambert Company

This is to certify that there has been presented to the

COMMISSIONER OF PATENTS AND TRADEMARKS

an application under 35 U.S.C. § 156 for an extension of the patent term. Since it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

1,213 days

from May 30, 2006, the original expiration date of the patent, subject to the provisions of 35 U.S.C. § 41(b), with all rights pertaining thereto as provided by 35 U.S.C. § 156(b).

I have caused the seal of the Patent and Trademark Office to be affixed this 15th day of July 1998.

Bruce A. Lehman

Bruce A. Lehman
Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks

ATTACHMENT E

US006569459B2

(12) **United States Patent**
Flashner-Barak

(10) **Patent No.: US 6,569,459 B2**
(45) **Date of Patent: May 27, 2003**

(54) **METHOD OF ADMINISTRATION OF PACLITAXEL-PLASMA PROTEIN FORMULATION**

(75) Inventor: **Moshe Flashner-Barak**, Petach Tikva (IL)

(73) Assignee: **Teva Pharmaceutical Industries, Ltd.**, Petah Tiqva (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/829,744

(22) Filed: Apr. 10, 2001

(65) **Prior Publication Data**

US 2001/0056070 A1 Dec. 27, 2001

Related U.S. Application Data

(60) Provisional application No. 60/195,912, filed on Apr. 10, 2000.

(51) Int. Cl.[7] A61K 9/14; A61K 9/50; A61F 2/00

(52) U.S. Cl. 424/489; 424/484; 424/486; 424/499; 424/400; 424/426

(58) Field of Search 424/489, 484, 424/401, 426, 486, 499; 514/449

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5,407,683	A		4/1995	Shively
5,415,869	A		5/1995	Straubinger et al.
5,470,311	A		11/1995	Setterstrom et al.
5,496,846	A		3/1996	Wilson et al.
5,504,102	A		4/1996	Agharkar et al.
5,580,575	A		12/1996	Unger et al.
5,621,001	A		4/1997	Canetta et al.
5,626,862	A		5/1997	Brem et al.
5,641,803	A		6/1997	Carretta et al.
5,651,986	A		7/1997	Brem et al.
5,684,169	A		11/1997	Hamada et al.
5,696,153	A	*	12/1997	Ainsworth et al. 514/449
5,846,565	A		12/1998	Brem et al.
5,888,530	A		3/1999	Netti et al.
6,071,952	A	*	6/2000	Owens et al. 514/449

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WO	WO 99/13914	*	3/1999

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Kuh HJ, Jang SH, Wientjes MG, Weaver JR, Au JL–S. Determinants of paclitaxel penetration and accumulation in human solid tumor. Journal of Pharmacol Exp Ther. Aug. 1999; 290:871–880.*

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(74) *Attorney, Agent, or Firm*—Kenyon & Kenyon

(57) **ABSTRACT**

The present invention provides for a method for treating human or animal patients with paclitaxel formulation, the method comprising an intratumoral dose of a paclitaxel formulation and an intravenous dose of paclitaxel. The intravenous dose occurs about 1 to about 7 days after the intratumoral dose. The paclitaxel formulation may typically be either a paclitaxel/HSA formulation or paclitaxel/γ-globulin formulation.

20 Claims, No Drawings

METHOD OF ADMINISTRATION OF PACLITAXEL-PLASMA PROTEIN FORMULATION

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of provisional application Ser. No. 60/195,912, filed Apr. 10, 2000, which is incorporated entirely herein by reference.

FIELD OF THE INVENTION

The present invention relates to the field of delivery of anti-tumor chemotherapeutics and more particularly to delivery of the anti-tumor chemotherapeutic, paclitaxel.

BACKGROUND

Paclitaxel is a high molecular weight (854 g/mole), highly lipophilic cytotoxic chemotherapeutic used as an anti-tumor agent in the treatment of carcinomas of the ovary, breast, lung and in the treatment AIDS related Karposi's sarcoma. Paclitaxel is currently used to treat breast cancer by pre-operatively administering the drug systemically. The pre-operation treatment reduces tumor burden prior to surgery, thus potentially improving the post-surgery prognosis. Although impressive success has been achieved using this approach, the treatment requires prolonged hospitalization and is accompanied by severe side-effects. Moreover, a significant number of cases (30%) do not result in a clinically satisfactory outcome either because the tumors are not reduced or because the side effects require that paclitaxel dosing be discontinued.

Paclitaxel's cytotoxic and anti-tumor properties derive from its ability to promote apoptosis (programed cell death) by inducing the assembly of microtubules from tubulin dimers and preventing microtubules from depolymerization. The stabilized microtubules inhibit normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic functions. In addition paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Paclitaxel Formulations

Paclitaxel is substantially water insoluble and must be administered using a solubilizing carrier. The currently approved paclitaxel carrier formulation, marketed as TAXOL®, comprising paclitaxel dissolved in ethanol and CREMOPHOR®EL (polyoxyethylated castor oil).

The TAXOL® carrier CREMOPHOR®EL can cause side effects, such as anaphylaxis and severe hyper-sensitivity. (Sarosy and Reed, *J Natl Med Assoc* (1993) 85(6):427–31.) To reduce the side effects, current recommended treatment with TAXOL® includes pre-medication with corticosteroids, diphenhydramine and H_2 antagonists.

Several alternative carriers have been proposed to address the anaphylaxis and severe hyper-sensitivity caused by the CREMOPHOR®EL. For example, U.S. Pat. No. 5,684,169, which is incorporated by reference, discloses unbranched cyclodextrin or branched cyclodextrin inclusion complexes of paclitaxel which improves the solubility of paclitaxel in water. The complex is produced by adding an unbranched cyclodextrin or a branched cyclodextrin to paclitaxel at a molar ratio of 1–20 times with respect to paclitaxel. By improving solubility, the cyclodextrin inclusion complex improves paclitaxel absorption in cancer patients.

U.S. Pat. No. 5,415,869, which is incorporated by reference, discloses paclitaxel or paclitaxel tumor-active analogs solubilized using one or more negatively charged phospholipids and one or more zwitterionic phospholipids. The phospholipid mixture entraps paclitaxel or the analog in a liposome. The liposome is in the form of particles having a size of 0.025 to 10 microns, with substantially no crystals of paclitaxel or the analog.

U.S. Pat. No. 5,580,575, which is incorporated by reference, discloses a therapeutic drug delivery system comprising gas-filled microspheres and a therapeutic drug, as well as, methods for employing such microspheres in therapeutic drug delivery. The preferred microspheres of the disclosure are gas-filled liposomes with an encapsulated drug. Methods of preparing such liposomes in drug delivery applications are also disclosed.

WO 99/13914, incorporated herein by reference, discloses that paclitaxel, and other slightly water soluble drugs can be formulated without CREMOPHOR®EL or other toxic solubilizers by forming a water soluble homogeneous complex with plasma proteins, such as human serum albumin (HSA) or human gamma globulin (γ-globulin). As disclosed by WO 99/13914 homogeneous aqueous solutions up to at least 4.68 mM paclitaxel (4 mg/mL) can be formulated using HSA. The plasma proteins act as a slow release depot of paclitaxel. WO 99/13914 further discloses a dosage range of paclitaxel-HSA complex containing 70–280 mg of paclitaxel per treatment. Such formulations can be made bio-equivalent to the conventional CREMOPHOR®EL containing formulations.

Other formulations for administering paclitaxel are disclosed in U.S. Pat. Nos. 5,504,102 and 5,407,683, incorporated herein by reference.

In addition, the slow infusion of CREMOPHOR®EL solutions has been studied as a means of avoiding or ameliorating the side effects of the CREMOPHOR®EL vehicle. The most common dosage is 135–175 mg/m^2 CREMOPHOR®EL, which is administered over a 3 hour, 6 hour, or 24 hour dosage schedule. (See U.S. Pat. Nos. 5,641,803, and 5,621,001, both incorporated herein by reference.) Other dosing schedules have been suggested to reduce toxic side effects, including 96 hour infusion every 21 days (U.S. Pat. No. 5,496,846, incorporated herein by reference) and 60–180 minutes, repeated a plurality of times during a 21 day period, each infusion separated by an interval of between 4 to 5 days. (U.S. Pat. No. 5,696,153, incorporated herein by reference).

Paclitaxel Chemotherapy Reservoir

An alternative method of administering paclitaxel is using a chemotherapy reservoir. U.S. Pat. Nos. 5,846,565, 5,626,862 and 5,651,986, which are incorporated by reference, discloses a method and devices for localized delivery of a chemotherapeutic agent to solid tumors, where the chemotherapeutic agent does not cross the blood-brain barrier and is characterized by poor bioavailability and/or short half-lives in vivo. The devices consist of reservoirs which release the chemotherapeutic over an extended period while at the same time preserving the bio-activity and bio-availability of the agent. The preferred embodiment is biodegradable polymeric matrices. Alternatively reservoirs can be made from non-biodegradable polymers or reservoirs connected to implanted infusion pumps. The devices are implanted within or immediately adjacent to the tumors to be treated or the site where tumors have been surgically removed. The patents further disclose the efficacy of paclitaxel and camptothecin delivered in polymeric implants prepared by compression molding of biodegradable and non-biodegradable polymers, respectively.

U.S. Pat. No. 5,888,530, which is incorporated by reference, discloses a method of enhancing the amount of a pharmaceutical composition delivered to a target tissue site in a mammal, by creating a transient differential between the hydrostatic pressure in the target site and a region near the target tissue site. An apparatus for performing the method is provided. In one form that apparatus includes a pharmaceutical reservoir, pump, and an agent reservoir and pump.

Chemotherapy reservoirs are also disclosed in U.S. Pat. No. 5,470,311 incorporated herein by reference.

Initial results testing such chemotherapy reservoirs have been disappointing. While a significantly lowered side effect profile has been demonstrated, there are no indications of clinical improvement.

The limitations of current chemotherapy reservoir technology is probably due to the retention of the chemotherapeutic drug only on the tumor periphery or at the injection site due to the poor penetration and distribution of the drug as a result of the neoplasm's high interstitial fluid pressure. A more potent anti-tumor effect can be achieved by targeting the chemotherapy directly to the tumor, i.e., intratumorally, rather than by systemic infusion.

We now report a method of delivering an anti-cancer chemotherapeutic, such as paclitaxel, by first administered paclitaxel by intratumoral injection and thereafter administering paclitaxel by intravenous injection. This invention takes advantage of the lower toxicity and side effects of paclitaxel/plasma solutions, and the ability of plasma proteins, such as HSA, to act as a slow release depot for paclitaxel.

SUMMARY OF THE INVENTION

The present invention provides for a method of delivering paclitaxel, the method comprising an intratumoral dose of a paclitaxel formulation and an intravenous infusion of paclitaxel wherein the intravenous infusion occurs about 24 hours to about 7 days after the intratumoral dose.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for a method of delivering a paclitaxel. According to the invention paclitaxel, as a paclitaxel formulation, is first brought into contact with substantially all the cells of a solid tumor, by an intratumoral dose. Thereafter paclitaxel is administered by intravenous infusion. The paclitaxel administered by intravenous infusion may be the same paclitaxel formulation used in the intratumoral dose. Alternatively, the paclitaxel may be administered by infusion of paclitaxel in any other soluble form.

While not being bound by theory, it is believed that the intratumoral dose of the paclitaxel formulation induces apoptosis within the tumor by slowly releasing paclitaxel into the tumor over a period of twenty-four hours to one week. The cell death that occurs within the tumor results in the collapses of the tumor structure. The collapsed tumor allows access of the second intravenous dose of paclitaxel to reach inside the partially collapsed tumor structure. One of skill in the art will recognize that, the invention is not limited to methods which function in this manner.

Intratumoral Dose of Paclitaxel Formulation

One aspect of the present invention provides for introducing a paclitaxel formulation intratumorally. For example, in one embodiment of the present invention, the intratumoral dose of paclitaxel formulation may be injected intratumorally using a syringe pump. The flow rate and pressure of the syringe pump will depend upon the tumor to be treated. The flow rate of the syringe pump may vary from about 0.0167 ml/min to about 0.5 ml/min. The preferred flow rate will deliver the paclitaxel formulation to greater than 90% of the tumor volume while delivering essentially no paclitaxel outside the tumor.

The paclitaxel formulation is preferably a soluble form of paclitaxel comprising a paclitaxel/plasma protein complex. As used herein, paclitaxel/plasma protein complex refers to paclitaxel in a water-ethanol solution containing a solubilizing amount of plasma protein wherein the paclitaxel forms a non-covalent complex with the plasma protein. Preferably the plasma protein is HSA or γ-globulin. Most preferably the plasma protein is HSA. One of skill in the art will understand that paclitaxel/plasma protein is not limited to the use of these two proteins and includes any plasma protein capable of forming a non-covalent paclitaxel/plasma protein complex and thereby solubilizing paclitaxel.

While not being bound by theory, it is proposed that administering a soluble form of paclitaxel, such as a paclitaxel/plasma protein complex, increases drug efficacy by promoting paclitaxel diffusion. Increased diffusion promotes apoptosis tumor cell death not only in the immediate zone of the injection but also at sites further into the tumor where the paclitaxel has migrated.

The mass of paclitaxel formulation delivered intratumorally depends upon the size of the tumor, and can range up to about 280 mg of paclitaxel. Preferably, the intratumoral mass of paclitaxel is from about 1 to about 60 mg of paclitaxel.

The volume of the dose is preferably about ¼ to about 1/12 the tumor volume. Most preferably the volume of the dose is about 1/10 of the tumor volume.

The preferred concentration of the paclitaxel formulation is about 4 to about 10 mg/ml of paclitaxel, or about 3.4 to about 8.5 mM paclitaxel.

Thus, a tumor with a 4 cm diameter has a volume of 33 cc. Consequently, a 6 ml of a 10 mg/ml dose of paclitaxel liquid delivered into the tumor results in a dose of 60 mg paclitaxel which is approximately the maximal intratumoral injection dose.

If the initial intratumoral administration of the paclitaxel formulation does not substantially shrink the solid tumor, an additional intratumoral dose of the paclitaxel formulation may be administered. The additional intratumoral dose may be at an identical, at a greater, or at a lower concentration of the paclitaxel formulation then the initial intratumoral dose. In one embodiment of the present invention, the paclitaxel/plasma protein may be administered by multiple intratumoral injections within a short period of time. The invention provides for multiple intratumoral injections of the paclitaxel formulation administered within 24 hours.

While not being bound by theory, administering the paclitaxel formulation by frequent intratumoral injections may increase the efficacy of paclitaxel by inducing apoptosis at various stages of the cell cycle.

Intravenous Infusion of Paclitaxel

Methods of delivering an anti-tumor chemotherapeutic by intravenous infusion are well known in the art and are described for example in U.S. Pat. Nos. 5,696,153, 5,496,846, and 5,641,803.

In one embodiment of the present invention, the paclitaxel is administered by intravenous infusion about twenty-four hours to about 1 week following the intratumoral dose as a

continuous infusion. The intravenous dose is typically administered over about 3 to about 12 hours. The paclitaxel administered by intravenous infusion may be the paclitaxel formulation used in the intratumoral dose administered as a saline solution of 5% dextrose or normal saline. Alternatively, the paclitaxel may be administered by infusion of paclitaxel in any other soluble form.

When the paclitaxel administered by intravenous infusion comprises the paclitaxel/plasma protein complex, the intratumoral or the intravenous treatment may be repeated in cycles. The administration of the paclitaxel/plasma protein complex may be repeated because of the decreased hypersensitivity reaction from the paclitaxel paclitaxel/plasma protein complex compared to TAXOL®.

In one embodiment, the intravenous infusion of paclitaxel comprises administering a plurality of repeated intravenous infusions subsequent to the intratumoral dose, wherein each infusion is separated by about seven days.

Another embodiment comprises administering an additional intravenous infusion of the paclitaxel formulation, about 4 to about 21 days subsequent to the intravenous infusion.

Another embodiment comprises an additional intratumoral dose administered subsequent to the intravenous dose. The additional intratumoral dose is preferably administered about 4 to about 21 days subsequent to the intravenous infusion.

In an alternative embodiment of the invention, the intravenous dose may be administered by solubilizing paclitaxel in CREMOPHOR®EL ethanol solutions. The solution of this embodiment comprises 6 mg/mL paclitaxel, corresponding to a paclitaxel concentration of about 7 mM, which is diluted prior to infusion with 0.9% sodium chloride injection, U.S.P, 5% dextrose injection, U.S.P, 5% dextrose and 0.9% sodium chloride injection, U.S.P, or 5% dextrose in Ringer's injection to a final concentration of 0.3 to 1.2 mg/mL. The maximum TAXOL® concentration which can be administered by intravenous infusion using this formulation is about 0.6 mg/mL.

The intravenous dose is preferably in the range of about 100 to about 200 mg/m^2. More preferably the intravenous dose is in the range of about 135 to about 175 mg/m^2.

The mass of paclitaxel administered by intravenous infusion is preferably between about about 70 to about 280 mg.

The function and advantage of these and other embodiments of the present invention will be more fully understood from the examples below. The following examples are intended to illustrate the benefits of the present invention, but do not exemplify the full scope of the invention.

EXAMPLES

EXAMPLE 1

Improved Spread of Evan's Blue-Albumin in a Human Mammary Adenocarcinoma MCF7 Xenograft in Immunocompromised Mice when Injecting Intratumorally Under Pressure as a Model for Paclitaxel/HSA Spread in the Tumor.

Purpose

The purpose of the study is to assess the efficiency of spread of a solution of Evan's blue dye—albumin complex in a tumor when injected intratumorally at different flow rates. The complex of the dye with albumin serves as a model of the complex of paclitaxel with albumin and allows visualization of the complex spread within the tumor.

Methods and Results

Nude (athymic mice) (~5 weeks of age) were injected subcutaneously with a cell suspension containing approximately 10^7 cells/0.1 ml of human mammary tumor cell line MCF7. On Day 28 following tumor cell implantation, all tumors were measured as described below, and the measurement recorded for each mouse as the pre-treatment baseline tumor volume. Tumor measurement were performed using calipers, to measure the tumor in two dimensions, at approximately 90°. to each other, at the longest and widest points. The tumor volume was calculated according to the formula, $(W^2 \times L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point.

Mice with tumor volumes within the range of 5–8 grams were allocated to the study. The mice were injected intratumorally with 1 ml of a solution of Evan's blue albumin in buffered saline using a Sage Instrument Model #355 syringe pump. The albumin dye complex serves as a visual model for the albumin paclitaxel complex. The solution was injected into the tumor at various flow rates between 0.0167 ml/min to 0.5 ml/min which corresponded to various back pressures. The faster the flow rate the higher the (not measured) back pressure is presumed to be. The flow rates tested were:

Flowrate
0.0167 ml/min (1 ml/60 min)
0.05 ml/min (1 ml/20 min)
0.1 ml/min (1 ml/10 min)
0.2 ml/min (1 ml/5 min)
0.5 ml/min (1 ml/2 min)

After the injections the mice were sacrificed, the tumor removed and the extent of the spread of the blue dye in the tumor measured visually.

From the results are given in the following table. One can see that the raising of the pressure results in a more efficient spread of the dye.

Flow rate	Percent of tumor volume dyed
0.0167 ml/min (1 ml/60 min)	2–5
0.05 ml/min (1 ml/20 min)	20–40
0.1 ml/min (1 ml/10 min)	40–60
0.2 ml/min (1 ml/5 min)	70–90
0.5 ml/min (1 ml/2 min)	>90

Conclusion

The results exemplify that the albumin can be effectively spread within the entire tumor volume when the pressure of the infusion is slightly raised. In our system, a flow rate of 0.2 ml/min suffices to raise the pressure and spread the soluble albumin complex. The efficient spread of the paclitaxel albumin complex results in more efficacious treatment of the solid tumors.

EXAMPLE 2

In Vivo Evaluation of the Anti-Tumor Effect of Intratumoral Injections of Paclitaxel/HSA in Human Breast Tumor (Cell line MCF7) Xenografts in Nude Mice, Purpose of Study

The purpose of the study is to assess the anti-tumor effect of intratumoral injections of Paclitaxel/HSA, a novel pro-

prietary compound of paclitaxel complexed with human serum albumin) against a human mammary tumor xenograft (cell line MCF7) in immunodeficient mice. The potential of paclitaxel/HSA to reduce tumor size is compared to the standard chemotherapeutic agent, TAXOL®.

Methods and Results

There are five study groups containing 6–10 mice per group. The mice are allocated to the following 5 groups:

Group Number	Drug	Dosage	Method of Administration	Number of Injections (within 24 hours)
I	No treatment (control)	—	—	—
II	Saline (control)	0.2 ml/gm[a]	Intratumoral	2
III	TAXOL®	0.2 ml/gm[a]	Intratumoral	2
IV	Paclitaxel/HSA	0.2 ml/gm[a]	Intratumoral	2
V	Paclitaxel/HSA	0.2 ml/gm[a]	Intratumoral (via high-pressure infusion)	2

[a]per gram tumor weight at 1 mg paclitaxel/ml

Nude (athymic mice) (~5 weeks of age) are injected subcutaneously with a cell suspension containing approximately 10^7 cells/0.1 ml of human mammary tumor cell line MCF7. The mice are examined routinely for the appearance of tumors. On Day 28 following tumor cell implantation, all tumors are measured as described below, and the measurement recorded for each mouse as the pre-treatment baseline tumor volume. Tumor measurement are performed using calipers, to measure the tumor in two dimensions, at approximately 90° to each other, at the longest and widest points. The tumor volume are calculated according to the formula, $(W^2 \times L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point.

All mice with tumor volumes within the range of 5–8 grams are allocated to study groups. Allocation to treatment groups are carried out based on the volume of the individual tumors, with each study group receiving an approximately equal representation of all tumor volumes. At study baseline, Day "0" of the Treatment Phase, all mice are receive the first injection according to their study group assignment. Approximately twenty-three hours later, the tumors are measured as described above, and the volumes recorded. Immediately following measurement, within 24 hours of the first injection, the mice are receive a second injection according to the study group assignment. Post-treatment tumor volumes are assessed at 48 hours, 7 days, 14 days, and 21 days following the initial injection. The mice are sacrificed and the tumors removed and weighed. The final weights for each treatment group are averaged and compared to the final weights obtained for the "no-treatment" group.

For each mouse within a study group, the post-treatment tumor volumes just before the 2nd injection at 24 hours, and at 48 hours, 7, 14 and 21 days following the initial injection, are measured and recorded. The relative tumor volume (post-treatment tumor volume/pre-treatment baseline tumor volume) are recorded at each time point, and the mean relative tumor volume for each time point, for all mice within a study group, are determined. Additionally, following sacrifice, the final weights for the tumors for each study group are averaged and compared to the final weights observed for the "no-treatment" group.

The expected results of the measurement of relative tumor volume (100xpost-treatment tumor volume/pre-treatment baseline tumor volume) (expected results) are tabulated in the following table:

Group	% tumor volume at 2 days	% tumor volume at 7 days	% tumor volume at 14 days	% tumor volume at 21 days
I	105	125	150	175
II	105	125	150	170
III	50	50	75	85
IV	40	40	60	75
V	40	25	25	40

Conclusion

Intratumoral injections of soluble paclitaxel/HSA are an effective method of affording tumor shrinkage. Two intratumoral injections separated by 24 hours are effective in shrinking the tumor to about 40% of its original value. Elevated pressure makes the injections more effective. Further injections, in an improved protocol, could conceivably bring about a full remission in the tumor.

What is claimed is:

1. A method of administering paclitaxel to a patient having a tumor, the method comprising;

introducing an intratumoral dose of a paclitaxel formulation; and,

subsequently providing an initial intravenous infusion of paclitaxel about 24 hours to about 7 days after the intratumoral dose.

2. The method of claim 1, wherein the paclitaxel formulation is a mixture of paclitaxel and plasma protein in an amount effective to solubilize the paclitaxel.

3. A method of treating a patient having a tumor comprising:

administering to a tumor at least one intratumoral dose of a first formulation comprising paclitaxel and a plasma protein, thereby inducing apoptosis; and

administering intravenously to a patient at least one dose of a second formulation comprising paclitaxel, the first dose of the second formulation occurring about 1 to about 7 days after the the intratumoral dose,

wherein the first and second formulations are the same or different, and

wherein the amount of plasma protein in the first formulation is an amount effective to solubilize the paclitaxel.

4. The method of claim 3, wherein the plasma protein is selected from the group consisting of human serum albumin and γ-globulin.

5. The method of claim 3, wherein the dose of paclitaxel formulation is between about 1 to about 60 mg of paclitaxel.

6. The method of claim 3, wherein the paclitaxel formulation is between about 4 to about 10 mg/ml paclitaxel.

7. The method of claim 3, wherein the intratumoral dose is administered by a plurality of injections of the paclitaxel formulation.

8. The method of claim 3, wherein the intratumoral dose of the paclitaxel formulation is administered by syringe pump.

9. The method of claim 3, wherein the intravenous infusion of paclitaxel comprises administering between about 70 to about 280 mg of paclitaxel.

10. The method of claim 3, wherein the intravenous infusion of paclitaxel comprises administering between about 100 to about 200 mg/m^2 of paclitaxel.

11. The method of claim 3, wherein the intravenous infusion of paclitaxel comprises administering between about 135 to about 175 mg/m^2 of paclitaxel.

12. The method of claim 3, wherein the intravenous infusion of paclitaxel comprises administering a mixture of

paclitaxel and plasma protein in an amount effective to solubilize the paclitaxel.

13. The method of claim 12, wherein the solubilizing plasma protein is selected from the group consisting of human serum albumin and γ-globulin.

14. The method of claim 1, wherein the intravenous infusion of paclitaxel comprises administering paclitaxel and polyoxyethylated castor oil.

15. The method of claim 3, wherein the intravenous infusion of paclitaxel comprises administering a plurality of intravenous infusions subsequent to the intratumoral dose.

16. The method of claim 3, further comprising administering an additional intravenous infusion of the paclitaxel formulation subsequent to the intravenous infusion.

17. The method of claim 16, wherein the additional intravenous dose is administered about 4 to about 21 days subsequent to the intravenous infusion.

18. The method of claim 16, further comprising administering an intratumoral dose of the paclitaxel formulation subsequent to the additional intravenous infusion.

19. The method of claim 3, further comprising administering an additional intratumoral dose of the paclitaxel formulation subsequent to the intravenous infusion.

20. The method of claim 19, wherein the additional intratumoral dose is administered about 4 to about 21 days subsequent to the intravenous infusion.

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